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(71) Applicant *(for all designated States except US)*: NOVARTIS AG [—/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HALLENBECK, Paul, L.** [US/US]; 7461 Rosewood Manor Lane, Gaithersburg, MD 20882 (US). **HAY, Carl, M.** [US/US]; 28301 Honeysuckle Drive, Damascus, MD 20872 (US). **GANESH, Shanthi** [LK/US]; 2 Townsend Street, Apt. 20209, San Francisco, CA 94107 (US). **POLICE, Seshidar, Reddy** [CA/US]; 157 Albacore Lane, Foster City, CA 94404 (US). **XU, Ling** [CN/US]; 18104 Dark Star Way, Boyds, MD 20841 (US). **YANG, Jingping** [CN/US]; 28 Redding Ridge Drive, North Potomac, MD 20878 (US). **CHENG, Cheng** [CN/US]; 12004 Montrose Village Terrace, Rockville, MD 20852 (US).

(74) Agent: **LOVE, Jane, M.**; Wilmer Cutler Pickering Hale and Dorr LLP, 300 Park Avenue, New York, NY 10022 (US).

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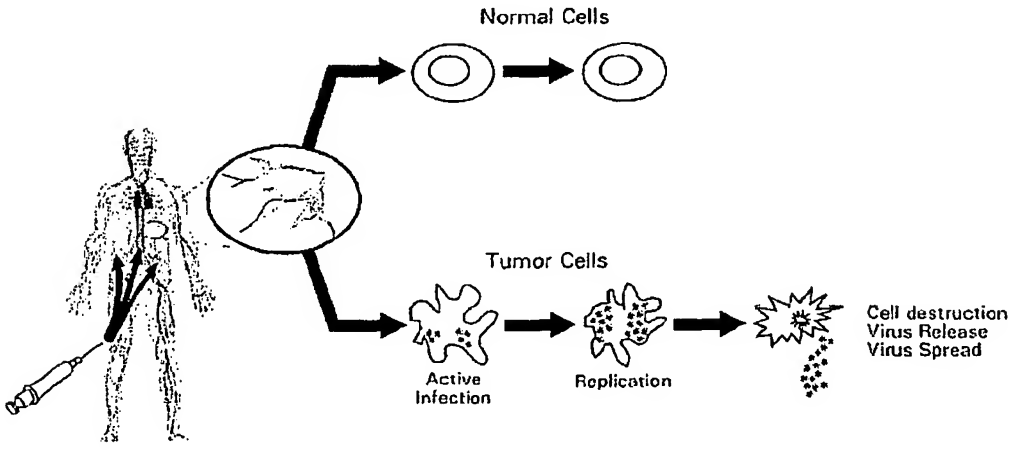
(54) Title: SENECA VALLEY VIRUS BASED COMPOSITIONS AND METHODS FOR TREATING DISEASE



(57) Abstract: The present invention relates to a novel RNA picornavirus that is called Seneca Valley virus ("SVV"). The invention provides isolated SVV nucleic acids and proteins encoded by these nucleic acids. Further, the invention provides antibodies that are raised against the SVV proteins. Because SVV has the ability to selectively kill some types of tumors, the invention provides methods of using SVV and SVV polypeptides to treat cancer. Because SVV specifically targets certain tumors, the invention provides methods of using SVV nucleic acids and proteins to detect cancer. Additionally, due to the information provided by the tumor-specific mechanisms of SVV, the invention provides methods of making new oncolytic virus derivatives and of altering viruses to have tumor-specific tropisms.

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Seneca Valley Virus Based Compositions and Methods for Treating Disease

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[0002] All patent applications, published patent applications, issued and granted patents, texts, and literature references cited in this specification are hereby incorporated herein by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

[0003] This application claims priority to U.S. Serial No. 60/506,182, which was filed on September 26, 2003, which is hereby incorporated in its entirety.

BACKGROUND OF THE INVENTION

[0004] Virotherapy holds great promise for treating cancer. Oncolytic viruses, which aim to specifically bind and kill cancer cells, whether native and/or engineered, may be more efficacious and less toxic than alternative treatments, such as chemotherapy and radiation. In addition, oncolytic virus therapy is the only therapy known that can amplify the therapeutic at the pharmacologically desired site.

[0005] A key aspect of cancer therapy is to achieve a high rate of killing of cancer cells versus normal cells. Accomplishing this goal has been difficult for many reasons, including the wide array of cell types involved, the systemic dissemination of cancer cells due to metastases, and the narrow biological differences between normal and cancer cells. While progress has been made, much still needs to be done to improve upon current cancer therapies.

[0006] In the past, surgeons have tried to remove tumors surgically without substantially harming the patient. Even complete removal of a primary tumor does not ensure survival since earlier metastases to unknown sites in the body are left undetected. There is also some research suggesting that surgical intervention may enhance the growth of distant metastases due to removal of tumor cells producing angiogenesis inhibitors. Finally, in many cases, the tumor grows back at the original site after surgical removal. Radiation aims to selectively destroy the most rapidly

proliferating cells at the expense of the others. However, tumor cells can escape radiation therapy either by becoming resistant or by being in a non-dividing state during treatment. In addition, radiation is not always selective in that many normal cells are actively dividing and killed by the treatment (gastrointestinal cells, hair follicles, etc.).

[0007] Like radiation, chemotherapy is not completely selective and thus destroys many normal cells, and does not kill all tumor cells due to drug resistance and/or division state of the cell. Thus, chemotherapy and radiation therapies exploit a small differential sensitivity that exists between normal and cancer cells, giving them a narrow therapeutic index. A small therapeutic index is clearly an undesirable property of any modality to treat cancer. Therefore, novel cancer therapeutic approaches overcoming these limitations are desired.

[0008] One such novel approach is oncolytic virus therapy. Initially, replication-defective viruses carrying cytotoxic transgenes were utilized in attempts to treat cancer. However, they were found to be inefficient in transduction of tumors and not adequately selective toward cancers. To overcome this limitation, viruses were either modified to replicate selectively in tumor cells or viruses were discovered to have natural tumor-selective properties. These oncolytic viruses thus had the properties to replicate, spread, and kill tumor cells selectively through a tumor mass by locally injecting the virus or by systemically delivering the virus (Figure 1).

[0009] Despite the early promise of this newly defined class of anti-cancer therapeutics, several limitations remain that may limit their use as a cancer therapeutic. Therefore, there is an ongoing need for novel oncolytic viruses that can be utilized for cancer therapy.

SUMMARY OF THE INVENTION

[0010] A novel RNA picornavirus has been discovered (hereafter referred to as Seneca Valley virus ("SVV")) whose native properties include the ability to selectively kill some types of tumors. As demonstrated below in the examples, SVV selectively kills tumor lines with neurotropic properties, in most cases with a greater than 10,000 fold difference in the amount of virus necessary to kill 50% of tumor cells versus normal cells (*i.e.*, the EC_{50} value). This result also translates *in vivo*, where

tumor explants in mice are selectively eliminated. Further, *in vivo* results indicate that SVV is not toxic to normal cells, in that up to $1x10^{14}$ vp/kg (vector or virus particles per kilogram) systemically administered causes no mortality and no visible clinical symptoms in immune deficient or immune competent mice.

[0011] SVV elicits efficacy at doses as low as $1x10^{8}$ vp/kg; therefore, a very high therapeutic index of >100,000 is achieved. Efficacy is very robust in that 100% of large pre-established tumors in mice can be completely eradicated (*see* Example 11). This efficacy may be mediated with a single systemic injection of SVV without any adjunct therapy. Furthermore, SVV injected mice show neither clinical symptoms nor recurrence of tumors for at least 200 days following injection. SVV can also be purified to high titer and can be produced at >200,000 virus particles per cell in permissive cell lines. SVV-based viral therapy therefore shows considerable promise as a safe, effective and new line of treatment for selected types of cancers. Further, SVV has a small and easily manipulatable genome, simple and fast lifecycle, and a well-understood capsid, and thus is amenable to modification. These properties, at least in part, allow for methods that generate modified SVVs that have new cell or tissue specific tropisms, such that SVV-based therapy can be directed to new tumor types resistant to infection by the original SVV isolate.

[0012] Accordingly, the present invention provides an isolated nucleic acid comprising a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or a contiguous portion of any one of these sequences that is at least 50 nucleotides in length, or 95% identical to a contiguous portion of any one of these sequences that is at least least 10, 15 or 20 nucleotides in length. The isolated nucleic acids of the invention can be RNA or DNA.

[0013] In other aspects, the invention provides an isolated nucleic acid that hybridizes under conditions of high, moderate stringency or low stringency to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length.

[0014] In another aspect, the invention provides a vector comprising a nucleic acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99%

sequence identity to SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length.

[0015] The present invention also provides an isolated polypeptide encoded by a nucleic acid having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to a nucleic acid sequence comprising SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or to a contiguous portion of any one of these sequences that is at least 50 nucleotides in length.

[0016] In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or to a contiguous portion of any one of these sequences that is at least 10 amino acids in length.

[0017] In another aspect, the invention provides an isolated antibody which specifically binds a polypeptide comprising an amino acid sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or to a contiguous portion of any one of these sequences that is at least 10 amino acids in length. The isolated antibody can be generated such that it binds to any protein epitope or antigen of SEQ ID NO:2. Further, the antibody can be a polyclonal antibody, a monoclonal antibody or a chimeric antibody.

[0018] In one aspect, the invention provides an isolated SVV or derivative or relative thereof, having a genomic sequence comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:1.

[0019] In another asepct, the invention provides an isolated virus having all the identifying characteristics and nucleic acid sequence of American Type Culture Collection (ATCC) Patent Deposit number PTA-5343. Some of the viruses of the present invention are directed to the PTA-5343 isolate, variants, homologues, relatives, derivatives and mutants of the PTA-5343 isolate, and variants, homologues, derivatives and mutants of other viruses that are modified in respect to sequences of

SVV (both wild-type and mutant) that are determined to be responsible for its oncolytic properties.

[0020] The present invention further provides an isolated SVV comprising the following characteristics: a single stranded RNA genome (positive (+) sense strand) of ~7.5 kilobases (kb); a diameter of ~27 nanometers (nm); a capsid comprising at least 3 proteins that have approximate molecular weights of about 31 kDa, 36 kDa and 27 kDa; a buoyant density of approximately 1.34 g/mL on cesium chloride (CsCl) gradients; and replication competence in tumor cells. In this aspect, the 31 kDa capsid protein (VP1) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:8; the 36 kDa capsid protein (VP2) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:4; and the 27 kDa capsid protein (VP3) can comprise an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:6.

[0021] In another aspect, the invention provides an isolated SVV derivative or relative comprising the following characteristics: replication competence in tumor cells, tumor-cell tropism, and lack of cytolysis in normal cells. In another aspect, the virus is replication competent in tumor cell types having neuroendocrine properties.

[0022] In other aspects, the present invention provides: a pharmaceutical composition comprising an effective amount of a virus of the invention and a pharmaceutically acceptable carrier; a cell comprising a virus of the invention; a viral lysate containing antigens of a virus of the invention; and an isolated and purified viral antigen obtained from a virus of the invention.

[0023] In yet another aspect, the invention provides a method of purifying a virus of the invention, comprising: infecting a cell with the virus; harvesting cell lysate; subjecting cell lysate to at least one round of gradient centrifugation; and isolating the virus from the gradient.

[0024] In another aspect, the invention provides a method for treating cancer comprising administering an effective amount of a virus or derivative thereof, so as to treat the cancer, wherein the virus has a genomic sequence that comprises a sequence

that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or to a portion of SEQ ID NO:1.

[0025] In another aspect, the invention provides a method for treating cancer comprising adminstering an effective amount of a virus comprising a capsid encoding region that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:3, 5, 7 or a contiguous portion thereof. The invention also provides a method for treating cancer comprising administering an effective amount of a virus comprising a capsid that comprises an amino acid sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:4, 6, 8 or a contiguous portion thereof.

[0026] In one aspect, the present invention provides a method for inhibiting cancer progression comprising contacting a cancer cell with a virus or derivative thereof, wherein the virus or derivative thereof specifically binds to the cancerous cell, wherein the virus has a genomic sequence that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21.

[0027] In another aspect, the present invention provides a method for killing cancer cells comprising contacting a cancer cell with an effective amount of a virus or derivative thereof, wherein the virus has a genomic sequence that comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21.

[0028] In these methods directed to cancer, the virus can be a picornavirus. The picornavirus can be a cardiovirus, erbovirus, aphthovirus, kobuvirus, hepatovirus, parechovirus, teschovirus, enterovirus or rhinovirus. The cardiovirus can be selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, encephalomyocarditis virus and SVV. The encephalomyocarditis virus can be selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626. The SVV can be a virus having the ATCC deposit number PTA-5343 or a virus comprising a nucleic acid sequence that is at least 65%, 70%, 75%,

80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:1 or a contiguous portion thereof.

[0029] The present invention also provides a method of killing an abnormally proliferative cell comprising contacting the cell with a virus of the invention. In one aspect, the abnormally proliferative cell is a tumor cell. In various aspects of this method, the tumor cell is selected from the group consisting of: human small cell lung cancer, human retinoblastoma, human neuroblastoma, human medulloblastoma, mouse neuroblastoma, Wilms' tumor, and human non-small cell lung cancer.

[0030] The present invention also provides a method of treating a neoplastic condition in a subject comprising administering to the subject an effective amount of a virus of the invention to the mammal. In one aspect, the neoplastic condition is a neuroendocrine cancer. In another aspect, the subject is a mammal. In another aspect, the mammal is a human.

[0031] The present invention also provides a method of producing a virus of the invention, comprising: culturing cells infected with the virus under conditions that allow for replication of the virus and recovering the virus from the cells or the supernatant. In one aspect of this method, the cells are PER.C6 cells. In another aspect of this method, the cells are H446 cells. In the various aspects of this method, the cells may produce over 200,000 virus particles per cell.

[0032] In another aspect, the present invention provides a method for detecting a virus of the invention, comprising: isolating RNA from test material suspected to contain the virus of the invention; labeling RNA corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1; probing the test material with the labeled RNA; and detecting the binding of the labeled RNA with the RNA isolated from the test material, wherein binding indicates the presence of the virus. In another aspect, the present invention provides a nucleic acid probe comprising a nucleotide sequence corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1 or its complement.

[0033] The present invention also provides a method for making an oncolytic cardiovirus, the method comprising: (a) comparing a SVV genomic sequence with a test virus genomic sequence; (b) identifying at least a first amino acid difference

between a polypeptide encoded by the SVV genomic sequence and a polypeptide encoded by the test virus genomic sequence; (c) mutating the test virus genomic sequence such that the polypeptide encoded by the test virus genomic sequence has at least one less amino acid difference to the polypeptide encoded by the SVV genomic sequence; (d) transfecting the mutated test virus genomic sequence into a tumor cell; and (e) determining whether the tumor cell is lytically infected by the mutated test virus genomic sequence. In one aspect, the amino acid(s) mutated in the test virus are amino acids in a structural region, such as in the capsid encoding region. In another aspect, the amino acids mutated in the test virus are amino acids in a non-structural region.

[0034] In one aspect of the method for making an oncolytic virus, the SVV genomic sequence is obtained from the isolated SVV having the ATCC deposit number PTA-5343 or from a virus comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof. In another aspect of this method, the step of mutating the test virus genomic sequence comprises mutating a cDNA having the test virus genomic sequence. In another aspect of this method, the step of transfecting the mutated test virus genomic sequence comprises transfecting RNA, wherein the RNA is generated from the cDNA having the mutated test virus genomic sequence.

[0035] In another aspect of the method for making an oncolytic cardiovirus, the test virus is a picornavirus. The test picornavirus can be a teschovirus, enterovirus, rhinovirus, cardiovirus, erbovirus, apthovirus, kobuvirus, hepatovirus, parechovirus or teschovirus. In another aspect, the test virus is a cardiovirus. In another aspect, the amino acid differences identified in the methods for making an oncolytic virus are between a SVV capsid protein and a test virus capsid protein sequence. In another aspect for making an oncolytic virus, the test virus genomic sequence is selected from the group consisting of: Vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus. In another aspect, the test virus genomic sequence is selected from an encephalomyocarditis virus. The encephalomyocarditis virus can be selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626. In yet another aspect, the encephalomyocarditis virus or any other

test virus can be selected from an isolate having a nucleic acid sequence comprising at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity to SVV of ATCC deposit number PTA-5343 or SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof.

[0036] In another aspect of the method for making an oncolytic cardiovirus, the amino acid difference between the test virus and SVV is in a capsid protein region of SVV, wherein the amino acid difference is aligned within SVV SEQ ID NO:4, 6 or 8.

[0037] The present invention also provides a method for making a mutant virus having an altered cell-type tropism, the method comprising: (a) creating a library of viral mutants comprising a plurality of nucleic acid sequences; (b) transfecting the library of viral mutants into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and (e) recovering a mutant virus that was produced in the non-permissive cell, thereby making a mutant virus having an altered tropism. In one aspect, this method further comprises the steps of: (f) incubating the recovered mutant virus in the non-permissive cell; and (g) recovering a mutant virus that that was produced in the non-permissive cell. In another aspect, the method further comprises iteratively repeating steps (f) and (g). In another aspect, the library of viral mutants is created from a parental sequence comprising SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof.

[0038] In one aspect of the method for making a mutant virus having an altered cell-type tropism, the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive cell-type in each well. In this aspect, the method can further comprise screening the platform to identify which wells contain a mutant virus that kills the cells. In another aspect, the screening is conducted by analyzing light absorbance in each well.

[0039] In another aspect of the method for making a mutant virus having an altered cell-type tropism, the non-permissive cell is a tumor cell.

[0040] In another aspect of the method for making a mutant virus having an altered cell-type tropism, the step of creating the library of viral mutants comprises: (i) providing a polynucleotide having a sequence identical to a portion of a genomic sequence of a virus; (ii) mutating the polynucleotide in order to generate a plurality of different mutant polynucleotide sequences; and (iii) ligating the plurality of mutated polynucleotides into a vector having the genomic sequence of the virus except for the portion of the genomic sequence of the virus that the polynucleotide in step (i) contains, thereby creating the library of viral mutants. In one aspect, the genomic sequence of a virus is from a picornavirus. In another aspect, the genomic sequence of a virus comprises a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof. In another aspect, in the step of creating the library of viral mutants, the mutating of step (ii) is conducted by random insertion of nucleotides into the polynucleotide. In one aspect, the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM). In another asepct, at least a portion of the nucleotides inserted into the polynucleotide encodes an epitope tag. In another aspect, in the step of creating the library of viral mutants, the mutating of step (ii) is conducted in a capsid encoding region of the polynucleotide.

[0041] The present invention also provides a method for making a mutant cardiovirus having an altered cell-type tropism, the method comprising: (a) creating a library of mutant polynucleotide sequences of a cardiovirus, wherein the creating comprises: providing a polynucleotide encoding a capsid region of the cardiovirus; mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and ligating the plurality of mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the cardiovirus except for the capsid-encoding region, thereby creating the library of mutant polynucleotide sequences of the cardiovirus; (b) transfecting the library of mutant polynucleotide sequences into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and (e) recovering a mutant virus that that was produced in the non-permissive cell, thereby making a mutant cardiovirus having an altered tropism. In one aspect, the method further

comprises the steps of: (f) incubating the recovered mutant virus in the non-permissive cell; and (g) recovering a mutant virus that that was produced in the non-permissive cell. In another aspect, the method further comprises iteratively repeating steps (f) and (g). In another aspect, the mutating is conducted by random insertion of nucleotides into the capsid-encoding polynucleotide. In another aspect, at least a portion of the nucleotides randomly inserted into the capsid-encoding polynucleotide encodes an epitope tag. In another aspect, the random insertion of nucleotides is conducted by TRIM. In another aspect, the plurality of different mutant capsid-encoding polynucleotide sequences comprises greater than 10^8 or 10^9different capsid-encoding polynucleotide sequences.

[0042] In one aspect, a method for making a mutant SVV having an altered cell-type tropism comprises: (a) creating a cDNA library of SVV mutants; (b) generating SVV RNA from the cDNA library of SVV mutants; (c) transfecting the SVV RNA into a permissive cell, such that a plurality of mutant SVV is produced; (d) isolating the plurality of mutant SVV; (e) incubating a non-permissive tumor cell with the isolated plurality of mutant SVV; and (f) recovering a mutant SVV that lytically infects the non-permissive tumor cell, thereby making a mutant SVV having an altered tropism. In another aspect, the method further comprises the steps of: (g) incubating the recovered mutant SVV in the non-permissive cell; and (h) recovering a mutant SVV that lytically infects the non-permissive tumor cell. In another apsect, the method further comprises iteratively repeating steps (g) and (h). In one aspect, the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive tumor cell-type in each well. In another aspect, the method further comprises screening the platform to identify which wells contain a mutant SVV that lytically infects the cells. In another aspect, the screening is conducted by analyzing light absorbance in each well. In one aspect, the cDNA library of SVV mutants comprises a plurality of mutant SVV capsid polynucleotide sequences. In another aspect, the plurality of mutant SVV capsid polynucleotide sequences is generated by random insertion of nucleotides. In another aspect, at least a portion of the sequence of the nucleotides randomly inserted encodes an epitope tag. In another aspect, the random insertion of nucleotides is conducted by TRIM. In another aspect, the cDNA library of SVV mutants is generated from a SVV

of ATCC deposit number PTA-5343. In another aspect, the cDNA library of SVV mutants is generated from a SVV comprising a sequence having at least 99%, 95%, 90%, 85%, 80%, 75%, 70%, or 65% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21. In another aspect, the non-permissive tumor cell is a tumor cell-line or a tumor cell-type isolated from a patient.

[0043] The present invention also provides a method for making a mutant virus having a tumor cell-type tropism *in vivo*, the method comprising: (a) creating a library of viral mutants comprising a plurality of nucleic acid sequences; (b) transfecting the library of viral mutants into a permissive cell, such that a plurality of mutant viruses is produced; (c) isolating the plurality of mutant viruses; (d) administering the isolated plurality of mutant viruses to a mammal with a tumor, wherein the mammal is not a natural host of the unmutated form of the mutant virus; and (e) recovering a virus that replicated in the tumor, thereby making a mutant virus having a tumor cell-type tropism *in vivo*. In one aspect, the step of creating a library of viral mutants comprises: providing a polynucleotide encoding a capsid region of a virus; mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and ligating the plurality of mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the virus except for the capsid-encoding region, thereby creating the library of viral mutants. In another aspect, the virus recovered in step (e) lytically infects cells of the tumor. In another aspect for a method for making a mutant virus having a tumor cell-type tropism *in vivo*, the tumor is a xenograft, a syngeneic tumor, an orthotopic tumor or a transgenic tumor. In another aspect, the mammal is a mouse.

[0044] For all the methods of the present invention, the virus can be a picornavirus. The picornavirus can be a cardiovirus, erbovirus, aphthovirus, kobuvirus, hepatovirus, parechovirus, teschovirus, entrovirus, or rhinovirus. The cardiovirus can be SVV. The SVV can be a SVV having the ATCC Patent Deposit No. PTA-5343 or a SVV comprising a sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO:1, 3, 6, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof. Further, the cardiovirus can be selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus. In one aspect, the

encephalomyocarditis virus is selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626. In another aspect, the present invention encompasses any virus that is selected from an isolate having at least 99%, 95%, 90%, 85%, 80%, 75%, 70%, or 65% sequence identity to SVV of ATCC deposit number PTA-5343 or SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or a contiguous portion thereof or is otherwise considered related to SVV to by sequence homology.

[0045] The present invention also provides an oncolytic virus made by any of the methods for making a mutant virus disclosed herein. In one aspect, the present invention provides a method for treating a patient with an oncolytic virus, the method comprising: (a) inactivating an oncolytic virus made by any of the methods for making a mutant virus disclosed herein, such that the oncolytic virus is non-infectious and the tropism of the oncolytic virus is unaffected; and (b) administering the irradiated oncolytic virus to a patient afflicted with a tumor. In another aspect, the method for treating a patient further comprises attaching a toxin to the inactivated oncolytic virus.

[0046] In another aspect, the present invention provides a method for treating a patient with a tumor with SVV, the method comprising: (a) inactivating a SVV such that the virus is non-infectious and the tropism is unaffected; and (b) administering the inactivated SVV in a patient afflicted with a tumor. In another aspect, the method for treating a patient with a tumor with SVV further comprises attaching a toxin to the inactivated SVV.

[0047] In another aspect, the present invention provides a SVV composition comprising an inactivated SVV. In another aspect, the present invention provides a SVV comprising an epitope tag incorporated in the capsid region.

[0048] The present invention also provides a method for treating a patient with a tumor with SVV, the method comprising: (a) creating a mutant SVV comprising an epitope tag encoded in the capsid; (b) attaching a toxin to the epitope tag; and (c) administering the mutant SVV with the attached toxin to a patient afflicted with a tumor. In one aspect, the creating comprises: inserting an oligonucleotide encoding an epitope tag into a capsid-encoding region polynucleotide

of SVV. In one aspect, the mutant SVV does not have an altered cell-type tropism. In another aspect, the method further comprises inactivating the mutant SVV such that the mutant SVV is not infectious.

[0049] The present invention also provides a method for detecting a tumor cell in a sample comprising: (a) isolating a tumor sample from a patient; (b) incubating the tumor sample with an epitope-tagged SVV; and (c) screening the tumor sample for bound SVV by detecting the epitope tag.

[0050] In one aspect, the present invention provides a method for detecting a tumor cell *in vivo* comprising: (a) administering to a patient an inactivated epitope-tagged SVV, wherein a label is conjugated to the epitope-tag; and (b) detecting the label in the patient. In the methods for detecting a tumor cell of the present invention, the SVV can be a mutant SVV generated by the methods disclosed herein.

[0051] Further, the present methods for treating neoplastic conditions, for detecting neoplastic conditions and for producing SVV, apply to wild-type SVV, mutant (including modified or variant) SVV, relatives of SVV, and other tumor-specific viruses of the invention.

[0052] The viruses of the present invention, and the compositions thereof, can be used in the manufacture of a medicament for treating the diseases mentioned herein. Further, the viruses and composition thereof of the invention can be used for the treatment of the diseases mentioned herein. Thus, in one aspect of the present invention, the present invention provides the use of SVV (or mutants, derivatives, relatives, and compositions thereof) for the treatment of cancer or in the manufacture of a medicament for treating cancer.

Deposit Information

[0053] The following material has been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia, 20110-2209, U.S.A., under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. All restrictions on the availability of the deposited material will be irrevocably removed upon the granting of a patent. Material: Seneca Valley Virus (SVV). ATCC Patent Deposit Number: PTA-5343. Date of Deposit: July 25, 2003.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] **Figure 1** shows a schematic of virotherapy using oncolytic viruses. Oncolytic viruses have the properties to replicate, spread and kill tumor cells selectively through a tumor mass by locally injecting the virus or by systemically delivering the virus.

[0055] **Figure 2** shows purified SVV stained with uranyl acetate and examined by transmission electron microscopy. Spherical virus particles are about 27 nm in diameter.

[0056] **Figure 3** is an electron micrograph of an SVV-infected PER.C6 cell that has a large crystalline inclusion and large vesicular bodies.

[0057] **Figure 4A** shows an analysis of SVV RNA. SVV genomic RNA is extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen Corp., Carlsbad, CA). RNA is resolved through a 1.25% denaturing agarose gel. The band is visualized by ethidium bromide (EtBr) staining and photographed. In lane 2, a predominant band of SVV genomic RNA is observed, indicating that the size of the full-length SVV genome is about 7.5 kilobases **[confirm]**.

[0058] **Figure 4B** is a schematic showing the genome structure and protein products generated from polyprotein processing for picornaviruses, including SVV.

[0059] **Figures 5A-5E** presents the nucleotide sequence of SVV (SEQ ID NO:1) and the encoded amino acid sequence (SEQ ID NO:2). The stop codon is depicted by a "*" at positions 5671-3. As a general note, in sequence disclosures that include positions where the exact nucleotide is being confirmed, these positions are represented by an "n". Therefore, in codons that possess an "n", the relevant amino acid is depicted by a "x".

[0060] **Figures 6A-6D** presents the nucleotide sequence (SEQ ID NO:1) of the majority of the full-length genome of SVV. The nucleotide sequence was derived from the SVV isolate having the ATCC Patent Deposit Number: PTA-5343. Date of Deposit: July 25, 2003.

[0061] **Figures 7A-7B** presents the amino acid sequence (SEQ ID NO:2) encoded by SEQ ID NO:1.

[0062] **Figure 8** presents the nucleotide sequence (SEQ ID NO:3) of the partial 1B or VP2 encoding region of SVV. This sequence is identical to nucleotides 4-429 of SEQ ID NO:1.

[0063] **Figure 9** presents the amino acid sequence (SEQ ID NO:4) of the partial SVV VP2 protein that is encoded by SEQ ID NO:3. The sequence listed in SEQ ID NO:4 is identical to amino acids 2-143 of SEQ ID NO:2.

[0064] **Figure 10** presents the nucleotide sequence (SEQ ID NO:5) of the 1C or VP3 encoding region of SVV. This sequence is identical to nucleotides 430-1146 of SEQ ID NO:1.

[0065] **Figure 11** presents the amino acid sequence (SEQ ID NO:6) of the SVV VP3 protein that is encoded by SEQ ID NO:5. The sequence listed in SEQ ID NO:6 is identical to amino acids 144-382 of SEQ ID NO:2.

[0066] **Figure 12** presents the nucleotide sequence (SEQ ID NO:7) of the 1D or VP1 encoding region of SVV. This sequence is identical to nucleotides 1147-1923 of SEQ ID NO:1.

[0067] **Figure 13** presents the amino acid sequence (SEQ ID NO:8) of the SVV VP1 protein that is encoded by SEQ ID NO:7. The sequence listed in SEQ ID NO:8 is identical to amino acids 383-641 of SEQ ID NO:2.

[0068] **Figure 14** presents the nucleotide sequence (SEQ ID NO:9) of the 2A encoding region of SVV. This sequence is identical to nucleotides 1924-1965 of SEQ ID NO:1.

[0069] **Figure 15** presents the amino acid sequence (SEQ ID NO:10) of the SVV 2A protein that is encoded by SEQ ID NO:9. The sequence listed in SEQ ID NO:10 is identical to amino acids 642-655 of SEQ ID NO:2.

[0070] **Figure 16** presents the nucleotide sequence (SEQ ID NO:11) of the 2B encoding region of SVV. This sequence is identical to nucleotides 1966-2349 of SEQ ID NO:1.

[0071] **Figure 17** presents the amino acid sequence (SEQ ID NO:12) of the SVV 2B protein that is encoded by SEQ ID NO:11. The sequence listed in SEQ ID NO:12 is identical to amino acids 656-783 of SEQ ID NO:2.

[0072] **Figure 18** presents the nucleotide sequence (SEQ ID NO:13) of the 2C encoding region of SVV. This sequence is identical to nucleotides 2350-3315 of SEQ ID NO:1.

[0073] **Figure 19** presents the amino acid sequence (SEQ ID NO:14) of the SVV 2C protein that is encoded by SEQ ID NO:13. The sequence listed in SEQ ID NO:14 is identical to amino acids 784-1105 of SEQ ID NO:2.

[0074] **Figure 20** presents the nucleotide sequence (SEQ ID NO:15) of the 3A encoding region of SVV. This sequence is identical to nucleotides 3316-3585 of SEQ ID NO:1.

[0075] **Figure 21** presents the amino acid sequence (SEQ ID NO:16) of the SVV 3A protein that is encoded by SEQ ID NO:15. The sequence listed in SEQ ID NO:16 is identical to amino acids 1106-1195 of SEQ ID NO:2.

[0076] **Figure 22** presents the nucleotide sequence (SEQ ID NO:17) of the 3B encoding region of SVV. This sequence is identical to nucleotides 3586-3651 of SEQ ID NO:1.

[0077] **Figure 23** presents the amino acid sequence (SEQ ID NO:18) of the SVV 3B protein that is encoded by SEQ ID NO:17. The sequence listed in SEQ ID NO:18 is identical to amino acids 1196-1217 of SEQ ID NO:2.

[0078] **Figure 24** presents the nucleotide sequence (SEQ ID NO:19) of the 3C encoding region of SVV. This sequence is identical to nucleotides 3652-4284 of SEQ ID NO:1.

[0079] **Figure 25** presents the amino acid sequence (SEQ ID NO:20) of the SVV 3C protein that is encoded by SEQ ID NO:19. The sequence listed in SEQ ID NO:20 is identical to amino acids 1218-1428 of SEQ ID NO:2.

[0080] **Figure 26** presents the nucleotide sequence (SEQ ID NO:21) of the 3D encoding region of SVV. This sequence is identical to nucleotides 4285-5673 of SEQ ID NO:1.

[0081] **Figure 27** presents the amino acid sequence (SEQ ID NO:22) of the SVV 3D protein that is encoded by SEQ ID NO:21. The sequence listed in SEQ ID NO:22 is identical to amino acids 1429-1890 of SEQ ID NO:2.

[0082] **Figures 28A-28H** present an amino acid sequence alignment between SVV SEQ ID NO:2 and various members of the *Cardiovirus* genus, such as Encephalomyocarditis virus (EMCV; species *Encephalomyocarditis virus*), Theiler's murine encephalomyocarditis virus (TMEV; species *Theilovirus*), a rat TMEV-like agent (TLV; species *Theilovirus*), and Vilyuisk human encephalomyelitis virus (VHEV; species *Theilovirus*). The specific sequences of the various Cardioviruses are presented in: SEQ ID NOs: 23 (EMCV-R), 24 (EMCV-PV21), 25 (EMCV-B), 26 (EMCV-Da), 27 (EMCV-Db), 28 (EMCV-PV2), 29 (EMCV-Mengo), 30 (TMEV/DA), 31 (TMEV/GDVII), 32 (TMEV/BeAn8386), 33 (TLV-NGS910) and 34 (VHEV/Siberia-55).

[0083] Number positions in Figure 28 do not correspond to the numbering of the sequence listings. The "/" symbol indicates cleavage sites where the polyprotein is cleaved into its final functional products. For example, the alignment between positions 1 and 157 is in the 1A (VP4) region. The alignment between positions: 159 and 428 is in the 1B (VP2) region; 430 and 668 is in the 1C (VP3) region; 670 and 967 is in the 1D (VP1) region; 969 and 1111 is in the 2A region; 1112 and 1276 is in the 2B region; 1278 and 1609 is in the 2C region; 1611 and 1700 is in the 3A region; 1702 and 1723 is in the 3B region; 1725 and 1946 is in the 3C region; 1948 and 2410 is in the 3D region. The alignment also shows regions of potential conservation or similarity between the viral sequences as can be determined by standard sequence analysis programs. The alignments were generated using BioEdit 5.0.9 and Clustal W 1.81.

[0084] **Figure 29** lists the final polypeptide products of SVV. Some conserved motifs are bolded and underlined: 2A/2B "cleavage" (NPGP); 2C ATP-binding (GxxGxGKS/T and hyhyhyxxD); 3B (VPg)/RNA attachment residue (Y); 3C (pro) active site residues (H, C, H); 3D (pol) motifs (KDEL/IR, PSG, YGDD, FLKR).

[0085] **Figure 30** lists the picornavirus species that were used in sequence analyses to determine the phylogenetic relationship between SVV and these picornaviruses (see Example 4).

[0086] **Figure 31** shows the phylogenetic relationship between SVV (SEQ ID NO:4) and other picornaviruses in view of VP2 sequence analyses. The figure shows a bootstrapped neighbor-joining tree (see Example 4).

[0087] **Figure 32** shows a bootstrapped neighbor-joinining tree for VP3 between SVV (SEQ ID NO:6) and other picornaviruses (see Example 4).

[0088] **Figure 33** shows a bootstrapped neighbor-joinining tree for VP1 between SVV (SEQ ID NO:8) and other picornaviruses (see Example 4).

[0089] **Figure 34** shows a bootstrapped neighbor-joinining tree for P1 (*i.e.*, 1A, 1B, 1C and 1D) between SVV (*i.e.*, partial P1 - amino acids 2-641 of SEQ ID NO:2) and other picornaviruses (see Example 4).

[0090] **Figure 35** shows a bootstrapped neighbor-joinining tree for 2C between SVV (SEQ ID NO:14) and other picornaviruses (see Example 4).

[0091] **Figure 36** shows a bootstrapped neighbor-joinining tree for 3C (pro) between SVV (SEQ ID NO:20) and other picornaviruses (see Example 4).

[0092] **Figure 37** shows a bootstrapped neighbor-joinining tree for 3D (pol) between SVV (SEQ ID NO:22) and other picornaviruses (see Example 4).

[0093] **Figure 38** presents the actual amino acid percent identities of VP2 between SVV (SEQ ID NO:4) and other picornaviruses (see Example 4).

[0094] **Figure 39** presents the actual amino acid percent identities of VP3 between SVV (SEQ ID NO:6) and other picornaviruses (see Example 4).

[0095] **Figure 40** presents the actual amino acid percent identities of VP1 between SVV (SEQ ID NO:8) and other picornaviruses (see Example 4).

[0096] **Figure 41** presents the actual amino acid percent identities of P1 between SVV (partial capsid or P1 - amino acids 2-641 of SEQ ID NO:2) and other picornaviruses (see Example 4).

[0097] **Figure 42** presents the actual amino acid percent identities of 2C between SVV (SEQ ID NO:14) and other picornaviruses (see Example 4).

[0098] **Figure 43** presents the actual amino acid percent identities of 3C (pro) between SVV (SEQ ID NO:20) and other picornaviruses (see Example 4).

[0100] **Figure 44** presents the actual amino acid percent identities of 3D (pol) between SVV (SEQ ID NO:22) and other picornaviruses (see Example 4).

[0101] **Figure 45** shows the VP2 (~36 kDa), VP1 (~31 kDa) and VP3 (~27 kDa) proteins of SVV as analyzed by SDS-PAGE. Purified SVV was subjected to SDS-PAGE and proteins were visualized by silver stain. Lane "MWt" is molecular weight markers; lane "SVV" contains structural proteins of SVV. The sizes of three molecular weight markers and the names of viral proteins are also given.

[0102] **Figures 46A-46B** show the amounts of SVV in blood and tumor following systemic administration (Example 7). H446 tumor bearing nude mice were treated with SVV at a dose of $1x10^{12}$ vp/kg by tail vein injection. The mice were bled at 0, 1, 3, 6, 24, 48, 72 hours and at 7 days post-injection, and the plasma was separated from the blood immediately after collection, diluted in infection medium, and used to infect PER.C6 cells. The tumors were harvested at 6, 24, 48, 72 hours and at 7 days post-injection. The tumors were cut into small sections and suspended in one mL of medium and CVL was made.

[0103] **Figures 46C-46D** presents data relating to SVV clearance *in vivo*. The figures show that SVV exhibits a substantially longer resident time in the blood compared to similar doses of i.v. adenovirus (Example 7), and therefore SVV has a slower clearance rate than adenovirus *in vivo*. Following a single intravenous (i.v.) dose, SVV remains present in the blood for up to 6 hours (Figure 46C; Figure 46C is a duplication of Figure 46A for comparison purposes to Figure 46D), whereas adenovirus is cleared or depleted from the blood in about an hour (Figure 46D).

[0104] **Figure 47** shows immunohistochemistry and hematoxylin and eosin (H&E) staining of H446 xenograft sections (Example 7). H446 tumor bearing nude mice were treated with Hank's balanced salt solution (HBSS) or SVV at a dose of $1x10^{12}$ vp/kg by tail vein injection. The mice were sacrified at 3 days post-injection and the tumors were collected. The virus proteins in the tumor cells are visualized by

immunohistochemistry using SVV-specific mouse antibodies (upper panels). The general morphology of H446 tumor cells collected from HBSS or SVV treated mice were stained by H&E stain (lower panels).

[0105] **Figure 48** shows SVV mediated cytotoxicity in primary human hepatocytes (Example 9). Primary human hepatocytes plated in collagen coated 12-well plates were infected with SVV at 1, 10 and 100 and 1000 particles per cell (ppc). Three days after infection, the cell associated lactate dehydrogenase (LDH) and LDH in the culture supernatant were measured separately. Percent cytotoxicity was determined as a ration of LDH units in supernatant over maximal cellular LDH plus supernatant LDH.

[0106] **Figure 49** shows virus production by SVV in selected cell lines. To assess the replicative abilities of SVV, selected normal cells and tumor cells were infected with SVV at one virus particle per cell (ppc) (Example 9). After 72 hours, cells were harvested and CVL was assayed for titer on PER.C6 cells. For each cell line, the efficiency of SVV replication was expressed as plaque forming units per milliliter (pfu/ml).

[0107] **Figure 50** shows toxicity in nude and CD1 mice according to body weights (Example 10). The mean body weight of mice in each treatment group were measured different days post virus administration. Mice were injected with a single dose of SVV or PBS by tail vein on day 1.

[0108] **Figure 51** shows efficacy in a H446 xenograft model. H446 tumors are established in nude mice and the mice are sorted into groups (n=10) and treated via tail vein injection with either HBSS or six different doses of SVV (Example 11). On study day 20, five mice from the HBSS group that bear large tumors (mean tumor volume = 2000 mm^3) were injected with $1x10^{11}$ vp/kg (indicated by an arrow). Data is expressed as mean tumor volume + standard deviation (SD).

[0109] **Figure 52** shows a picture of H446 xenograft nude mice that have been untreated or treated with SVV (Example 11). The efficacy of SVV is very robust in that 100% of large pre-established tumors were completely eradicated. SVV-treated mice show neither clinical symptoms nor recurrence of tumors for at least 200 days following injection.

[0110] **Figure 53** presents data relating to SVV tumor specificity and efficacy *in vitro* (Example 11). The graphs show cell survival following incubation of either H446 human small cell lung carcinoma (SCLC) tumor cells (top graph) or normal human H460 cells (bottom graph). SVV specifically killed the tumor cells with an EC_{50} of approximately 10^{-3} particles per cell. In contrast, normal human cells were not killed at any concentration of SVV.

[0111] **Figure 54** depicts a representative plasmid containing the complete genome of SVV (Example 15). The presence of the T7 promoter on the vector upstream of the SVV sequence allows for the *in vitro* transcription of the SVV sequence such that SVV RNA molecules can be generated.

[0112] **Figure 55** depicts a schematic for the construction of a full-length and functional genomic SVV plasmid and subsequent SVV virus production (Example 16). To obtain a functional genomic SVV clone, the complete genome of a SVV can be cloned into a vector with a T7 promoter. This can be accomplished by making cDNA clones of the virus, sequencing them and cloning contiguous pieces into one plasmid, resulting in the plasmid depicted "pSVV". The plasmid with the full genome of SVV can then be reverse-transcribed to generate SVV RNA. The SVV RNA is then transfected into permissive mammalian cells and SVV virus particles can then be recovered and purified.

[0113] **Figure 56** depicts a schematic for the construction of a vector ("pSVV capsid") containing the coding sequence (*i.e.*, coding regions for 1A-1D) for the SVV capsid (Example 16). The pSVV capsid can then be used to generate a library of SVV capsid mutants.

[0114] **Figure 57** shows one method of mutating the SVV capsid for the generation of a library of SVV capsid mutants (Example 16). The figure illustrates the insertion of an oligonucleotide sequence at random sites of the plasmid. The oligonucleotides can be random oligonucleotides, oligonucleotides with known sequences, or an oligonucleotide encoding an epitope tag. In the figure, the restriction enzyme CviJI randomly cleaves the pSVV capsid DNA. Linearized pSVV capsid DNA that has been cut at a single site is isolated and purified from a gel, and ligated with oligonucleotides.

[0115] **Figure 58** presents a scheme to generate a library of full-length SVV mutants comprising sequence mutations in the capsid encoding region (Example 16). For example, the capsid encoding region from a pSVV capsid mutant library (generated according to the scheme depicted in Figure 57, for example) is isolated by restriction digestion and gel purification. The vector containing the full-length SVV sequence is also digested such that the capsid encoding region is cut out. The capsid encoding region from the pSVV capsid mutant library is then ligated to the pSVV vector that is missing its wild-type capsid sequence, thereby generating a library of full-length SVV mutants (the "pSVVFL" vector) having a plurality of mutations in the capsid encoding region.

[0116] **Figure 59** presents a general method for producing the SVV virus particles comprising a library of capsid mutations (Example 16). The pSVVFL vector is reverse-transcribed to generate SVV RNA. The SVV RNA is transfected into permissive cells, wherein SVV mutant virus particles are produced. The virus particles lyse the cells and a population of SVV virus particles comprising a plurality of capsid variants, "SVV capsid library," are isolated.

[0117] **Figure 60** shows a general method for screening SVV capsid mutants that can specifically infect tumor cells while being unable to infect non-tumor cells. The SVV capsid library is incubated with a tumor cell line or tissue of interest. After an initial incubation period, the cells are washed such that SVV virus particles that were unable to gain entry into the cells are eliminated. The cells are then maintained in culture until viral lysis is observed. Culture supernatant is then collected to isolate SVV capsid mutants that were able to lytically infect the tumor cell. These viruses can then be grown-up by infecting a known permissive cell-line prior to a counter-screen. A counter-screen is performed by incubating the SVV capsid mutant viruses that were able to infect the tumor cell with normal cells. Only those viruses that remain unbound in the supernatant are collected, thereby isolating mutant SVV viruses that have tumor-specificity. This process can be repeated to further refine the isolation of SVV tumor-specific viruses.

[0118] **Figure 61** shows a traditional method for testing whether virus mutants can bind and/or infect cell lines. Traditional methods require what are often inefficient methods for growing cell-lines, *i.e.* flasks, such that a mass-screen of a

library of virus mutants in relation to a number of different cell-lines becomes burdensome.

[0119] **Figure 62** shows a high-throughput method of the invention for screening virus mutants that have the ability to specifically infect different cell-lines (Example 16). In this figure, a number of different tumor cell-lines are grown in a 384 well-plate. To each well, a sample of a virus is added (for example, a sample of a SVV capsid library). From those wells which show cytopathic effects, the media is collected such that any viruses in the media can be amplified by infecting permissive cell lines (for example, for SVV, H446 or PER.C6) in flasks or large tissue culture plates. The viruses are grown such that the RNA can be isolated and the sequence analyzed to determine the encoded peptide sequence inserted by the oligonucleotide-insertion mutagenesis of the capsid. The peptide itself can then be tested to determine whether it can bind to a tumor cell-type specifically.

[0120] **Figure 63** shows another high-throughput screening schematic (Example 16). Tumor and normal cell lines are grown in multi-well plates. Viruses are added to each well to test whether the cells are killed by virus-mediated lysis. Cytopathic effects can be quickly assayed by reading the light-absorbance in each well. Viruses from the wells showing cytopathic effects are grown up and tested in further *in vitro* (re-testing of tumor and normal cell lines) and *in vivo* models (testing whether the virus can kill explanted tumors in mice).

[0121] **Figure 64** shows that SVV capsid mutants having new tumor-specific tropisms can be analyzed to generate tumor-selective peptides. Those SVV capsid mutants that enable the specific infection of a tumor cell line are sequenced to determine the peptide encoded by the oligonucleotide insertion. An amino acid consensus sequence can thereby be determined from the successful capsid mutants. Peptides having the consensus sequence can then be tested to determine whether they can bind specifically to the tumor cell-type in question. Tumor-selective peptides can then be attached to toxins or drugs in order to serve as tumor-specific targeting vehicles.

[0122] **Figure 65** illustrates that an SVV capsid library can be first tested *in vivo*. Mice (including normal, athymic, nude, CD-1 transgenics, etc.) can be

explanted with a specific tumor. These mice are then injected with a SVV derivative library, such as a SVV capsid library. At certain time points, tumor cells are recovered from the mice, such that in those mice that display the elimination of a tumor, viruses will be isolated from initial tumor samples and grown-up in permissive cell lines.

[0123] **Figure 66** shows a clinical testing program for the SVV derivatives of the present invention.

[0124] **Figure 67** illustrates that SVV derivatives (with new tumor tropisms) encoding epitope tags in their capsid can be used for a variety of purposes. They can be used as a screening reagent to detect whether a specific tumor cell is present in tissue samples by assaying for the presence of the epitope. Alternatively, toxins or other therapeutics can be attached to the epitope tag, and the virus then administered to patients. Further, wild-type or derivative SVV can be irradiated or inactivated such that the virus particle itself is used as a therapeutic device. Either the virus particle induces cellular apoptosis due to the presence of apoptosis-inducing peptides, or the particle can have a toxin or some other therapeutic attached such that the virus is used a specific-targeting delivery device.

[0125] **Figure 68** shows the basic life-cycle of the picornavirus.

[0126] **Figure 69** shows a comparison of the polypeptide lengths of SVV compared to other picornaviruses.

[0127] **Figure 70** lists an amino acid comparison of the picornavirus 2A-like NPG/P proteins. The sequence for SVV is listed at residues 635-656 of SEQ ID NO:2.

[0128] **Figure 71** lists the amino acid sequence (SEQ ID NO:23) for EMCV-R.

[0129] **Figure 72** lists the amino acid sequence (SEQ ID NO:24) for EMCV-PV21 (Accession CAA52361).

[0130] **Figure 73** lists the amino acid sequence (SEQ ID NO:25) for EMCV-B (Accession P17593).

[0131] **Figure 74** lists the amino acid sequence (SEQ ID NO:26) for EMCV-Da (Accession P17594).

[0132] **Figure 75** lists the amino acid sequence (SEQ ID NO:27) for EMCV-Db.

[0133] **Figure 76** lists the amino acid sequence (SEQ ID NO:28) for EMCV-PV2 (Accession CAA60776).

[0134] **Figure 77** lists the amino acid sequence (SEQ ID NO:29) for EMCV-mengo (Accession AAA46547).

[0135] **Figure 78** lists the amino acid sequence (SEQ ID NO:30) for TMEV/DA (Accession AAA47928).

[0136] **Figure 79** lists the amino acid sequence (SEQ ID NO:31) for TMEV/GDVII (Accession AAA47929).

[0137] **Figure 80** lists the amino acid sequence (SEQ ID NO:32) for TMEV/BeAn8386 (Accession AAA47930).

[0138] **Figure 81** lists the amino acid sequence (SEQ ID NO:33) for TLV-NGS910 (Accession BAC58035).

[0139] **Figure 82** lists the amino acid sequence (SEQ ID NO:34) for VHEV/Siberia-55 (Accession AAA47931).

DETAILED DESCRIPTION OF THE INVENTION

[0140] The terms "virus," "viral particle," "virus particle," "vector particle," "viral vector particle," and "virion" are used interchangeably and are to be understood broadly - for example - as meaning infectious viral particles that are formed when, *e.g.*, a viral vector of the invention is transduced or transfected into an appropriate cell or cell line for the generation of infectious particles.

[0141] The terms "derivative," "mutant," "variant" and "modified" are used interchangeably to generally indicate that a derivative, mutant, variant or modified virus can have a nucleic acid or amino acid sequence difference in respect to a template viral nucleic acid or amino acid sequence. For example, a SVV derivative, mutant, variant or modified SVV may refer to a SVV that has a nucleic acid or amino

acid sequence difference with respect to the wild-type SVV nucleic acid or amino acid sequence of ATCC Patent Deposit Number PTA-5343.

[0142] As used herein, the terms "cancer," "cancer cells," "neoplastic cells," "neoplasia," "tumor," and "tumor cells," are used interchangeably, and refer to cells that exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Neoplastic cells can be malignant or benign. According to the present invention, one type of preferred tumor cells are those with neurotropic properties.

[0143] The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm such as Protein-Protein BLAST (Protein-Protein BLAST of GenBank databases (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410)) or by visual inspection. The BLAST algortihm is described in Altschul *et al.*, J. Mol. Biol., 215:403-410 (1990), and publicly available BLAST software is provided through the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/).

[0144] For example, as used herein, the term "at least 90% identical to" refers to percent identities from 90 to 100 relative to the reference polypeptides (or polynucleotides). Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared, no more than 10% (*i.e.*, 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptide. Similar comparisons can be made between a test and reference polynucleotide. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, *e.g.*, 10 out of 100 amino acid difference (90% identity). Differences are defined as nucleic acid or amino acid substitutions, insertions or deletions. At the level of identities above about 85-90%, the result should be

independent of the program and gap paramaters set; such high levels of identity can be assessed readily, often without relying on software.

[0145] In the context of the present invention, the term "isolated" refers to a nucleic acid molecule, polypeptide, virus or cell that, by the hand of man, exists apart from its native environment. An isolated nucleic acid molecule or polypeptide may exist in a purified form or may exist in a non-native environment, such as, for example, a recombinant host cell. An isolated virus or cell may exist in a purified form, such as in a cell culture, or may exist in a non-native environment such as, for example, a recombinant or xenogeneic organism.

[0146] The term "naturally occurring" or "wildtype" is used to describe an entity that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

[0147] The concepts of "high stringency," "intermediate stringency" or "low stringency" refer to nucleic acid hybridization conditions. High stringency conditions refers to conditions that require a greater identity between a target's nucleic acid sequence and a probe's nucleic acid sequence in order for annealing or hybridization to occur between the target and the probe. Low stringency conditions refer to conditions that require a lower identity between a target's nucleic acid sequence and a probe's nucleic acid sequence in order for annealing or hybridization to occur between the target and the probe. Stringency conditions can be controlled by the salt concentration of the buffer or by the temperature at which the hybridization is carried out, where higher salt concentrations result in less stringent conditions and where higher temperatures result in more stringent conditions. Although stringency conditions will vary based on the length and nucleic acid content of the sequences undergoing hybridization, representative conditions of high, intermediate and low stringency are described in the following exemplary conditions. A commonly used hybridization buffer is SSC (sodium chloride sodium citrate) with a 20X stock concentration corresponding to 0.3 M trisodium citrate and 3 M NaCl. For high stringency conditions, the working concentration of SSC can be 0.1X – 0.5X (1.5 – 7.5 mM trisodium citrate, 15 – 75 mM NaCl) with the hybridization temperature set at

65°C. Intermediate conditions typically utilize a 0.5X – 2X SSC concentration (7.5 – 30 mM trisodium citrate, 75 – 300 mM NaCl) at a temperature of 55 – 62°C. Hybridizations conducted under low stringency conditions can use a 2X – 5X SSC concentration (30 – 75 mM trisodium citrate, 300 – 750 mM NaCl) at a temperature of 50 – 55°C. Note that these conditions are merely exemplary and are not to be considered limitations.

Seneca Valley Virus (SVV):

[0148] SVV is a novel, heretofore undiscovered RNA virus, most closely related to members from the *Cardiovirus* genus in the *Picornaviridae* family. Thus, for purposes of the present invention, SVV is considered to be a member of the *Cardiovirus* genus and the *Picornaviridae* family. Cardioviruses are distinguished from other picornaviruses by special features of their genome organization, common pathological properites, and the dissociability of their virions at pHs between 5 and 7 in 0.1M NaCl (Scraba, D. *et al.*, "Cardioviruses (*Picornaviridae*)," in Encyclopedia of Virology, 2nd Edition, R.G. Webseter and A. Granoff, Editors, 1999). The results of sequence analyses between SVV and other Cardioviruses are discussed hereinbelow.

[0149] The genome of SVV consists of one single-stranded positive (+) sense strand RNA molecule having a predicted size of about 7.5 kb (see Figure 4A). As SVV is a picornavirus, it has a number of features that are conserved in all picornaviruses: (i) genomic RNA is infectious, and thus can be transfected into cells to bypass the virus-receptor binding and entry steps in the viral life cycle; (ii) a long (about 600-1200 bp) untranslated region (UTR) at the 5' end of the genome and a shorter 3' untranslated region (about 50-100 bp); (iii) the 5' UTR contains a clover-leaf secondary structure known as the internal ribosome entry site (IRES); (iv) the rest of the genome encodes a single polyprotein and (v) both ends of the genome are modified, the 5' end by a covalently attached small, basic protein, "Vpg," and the 3' end by polyadenylation.

[0150] The present invention provides the isolated SVV virus (ATCC Patent Deposit number PTA-5343) and the complete genomic content of SVV therefrom. Presently, the largest SVV genomic fragment that has been sequenced is an isolated SVV nucleic acid, derived from the PTA-5343 isolate, that comprises the majority of

the SVV genomic sequence, and is listed in Figures 5A-5E and Figures 6A-6D, and has the designation of SEQ ID NO:1 herein. Translation of this nucleotide sequence shows that the majority of the single polyprotein of SVV is encoded by SEQ ID NO:1. The amino acid sequence encoded by nucleotides 1 to 5673 of SEQ ID NO:1 is listed in Figures 5A-E and Figures 7A-7B has the designation of SEQ ID NO:2 herein. The present invention therefore provides isolated portions of SEQ ID NO:1, including SEQ ID NOs:3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, that can be subcloned into expression vectors such that polypeptides encoded by these portions of SEQ ID NO:1 can be isolated. Further encompassed by the invention are isolated nucleic acids that can hybridize to SEQ ID NO:1, or any portion thereof, under high, moderate or low stringency conditions.

[0151] The present invention also provides an isolated partial SVV VP2 (1B) protein with the amino acid sequence of SEQ ID NO:4, as listed in Figure 9 (which corresponds to amino acids 2-143 of SEQ ID NO:2). The amino acid sequence of the partial SVV VP2 protein is encoded by the nucleic acid sequence of SEQ ID NO:3, as listed in Figure 8 (which corresponds to nucleotides 4-429 of SEQ ID NO:1).

[0152] The present invention also provides an isolated SVV VP3 (1C) protein with the amino acid sequence of SEQ ID NO:6, as listed in Figure 11 (which corresponds to amino acids 144-382 of SEQ ID NO:2). The amino acid sequence of the SVV VP3 protein is encoded by the nucleic acid sequence of SEQ ID NO:5, as listed in Figure 10 (which corresponds to nucleotides 430-1146 of SEQ ID NO:1).

[0153] The present invention also provides an isolated SVV VP1 (1D) protein with the amino acid sequence of SEQ ID NO:8, as listed in Figure 13 (which corresponds to amino acids 383-641 of SEQ ID NO:2). The amino acid sequence of the SVV VP1 protein is encoded by the nucleic acid sequence of SEQ ID NO:7, as listed in Figure 12 (which corresponds to nucleotides 1147-1923 of SEQ ID NO:1).

[0154] The present invention also provides an isolated SVV 2A protein with the amino acid sequence of SEQ ID NO:10, as listed in Figure 15 (which corresponds to amino acids 642-655 of SEQ ID NO:2). The amino acid sequence of the SVV 2A protein is encoded by the nucleic acid sequence of SEQ ID NO:9, as listed in Figure 14 (which corresponds to nucleotides 1924-1965 of SEQ ID NO:1).

[0155] The present invention also provides an isolated SVV 2B protein with the amino acid sequence of SEQ ID NO:12, as listed in Figure 17 (which corresponds to amino acids 656-783 of SEQ ID NO:2). The amino acid sequence of the SVV 2B protein is encoded by the nucleic acid sequence of SEQ ID NO:11, as listed in Figure 16 (which corresponds to nucleotides 1966-2349 of SEQ ID NO:1).

[0156] The present invention also provides an isolated SVV 2C protein with the amino acid sequence of SEQ ID NO:14, as listed in Figure 19 (which corresponds to amino acids 784-1105 of SEQ ID NO:2). The amino acid sequence of the SVV 2B protein is encoded by the nucleic acid sequence of SEQ ID NO:13, as listed in Figure 18 (which corresponds to nucleotides 2350-3315 of SEQ ID NO:1).

[0157] The present invention also provides an isolated SVV 3A protein with the amino acid sequence of SEQ ID NO:16, as listed in Figure 21 (which corresponds to amino acids 1106-1195 of SEQ ID NO:2). The amino acid sequence of the SVV 3A protein is encoded by the nucleic acid sequence of SEQ ID NO:15, as listed in Figure 20 (which corresponds to nucleotides 3316-3585 of SEQ ID NO:1).

[0158] The present invention also provides an isolated SVV 3B (VPg) protein with the amino acid sequence of SEQ ID NO:18, as listed in Figure 23 (which corresponds to amino acids 1196-1217 of SEQ ID NO:2). The amino acid sequence of the SVV 3B protein is encoded by the nucleic acid sequence of SEQ ID NO:17, as listed in Figure 22 (which corresponds to nucleotides 3586-3651 of SEQ ID NO:1).

[0159] The present invention also provides an isolated SVV 3C ("pro" or "protease") protein with the amino acid sequence of SEQ ID NO:20, as listed in Figure 25 (which corresponds to amino acids 1218-1428 of SEQ ID NO:2). The amino acid sequence of the SVV 3C protein is encoded by the nucleic acid sequence of SEQ ID NO:19, as listed in Figure 24 (which corresponds to nucleotides 3652-4284 of SEQ ID NO:1).

[0160] The present invention also provides an isolated SVV 3D ("pol" or "polymerase") protein with the amino acid sequence of SEQ ID NO:22, as listed in Figure 27 (which corresponds to amino acids 1429-1890 of SEQ ID NO:2). The amino acid sequence of the SVV 3C protein is encoded by the nucleic acid sequence of SEQ ID NO:19, as listed in Figure 24 (which corresponds to nucleotides 4285-

5673 of SEQ ID NO:1; nucleotides 5671-5673, "tga," code for a stop-codon, which is depicted in the amino acid sequence listings as an asterisk "*").

[0161] The nucleic acids of the present invention include both RNA and DNA forms, and implicitly, the complementary sequences of the provided listings.

[0162] Thus, the isolated SVV nucleic acid depicted by SEQ ID NO:1 has a length of 5,752 nucleotides that encodes a polypeptide with the amino acid sequence depicted by SEQ ID NO:2. The SVV genomic sequence is translated as a single polyprotein that is cleaved into various downstream "translation products." The present invention encompasses all nucleic acid fragments of SEQ ID NO:1 and all polypeptides encoded by such fragments.

[0163] The majority of the full-length SVV polyprotein amino acid sequence is encoded by nucleotides 1-5673 of SEQ ID NO:1. The polyprotein is cleaved into three precursor proteins, P1, P2 and P3 (see Figure 4B). P1, P2 and P3 are further cleaved into smaller products. The cleavage products of the structural region P1 (1ABCD; or the capsid region) are 1ABC, VP0, VP4, VP2, VP3 and VP1. The cleavage products of the non-structural protein P2 (2ABC) are 2A, 2BC, 2B and 2C. The cleavage products of the non-structural region P3 polyprotein (3ABCD) are 3AB, 3CD, 3A, 3C, 3D, 3C', and 3D'. In certain embodiments, the present invention provides isolated nucleic acids that comprise: (i) the sequence of 2ABC (nucleotides 1924-3315 of SEQ ID NO:1) and the protein encoded therefrom; (ii) the sequence of 2BC (nucleotides 1966-3315 of SEQ ID NO:1) and the protein encoded therefrom; (iii) the sequence of 3ABCD (nucleotides 3316-5673 of SEQ ID NO:1) and the protein encoded therefrom; (iv) the sequence of 3AB (nucleotides 3316-3651 of SEQ ID NO:1) and the protein encoded therefrom; and (v) the sequence of 3CD (nucleotides 3652-5673 of SEQ ID NO:1) and the protein encoded therefrom.

[0164] The basic capsid structure of picornaviruses consists of a densely packed icosahedral arrangement of 60 protomers, each consisting of 4 polypeptides, VP1, VP2, VP3 and VP4, all of which are derived from the cleavage of the original protomer, VP0. The SVV virus particle is about 27 nm in diameter (*see* Figure 2), which is consistent with the size of other picornavirus particles, which are about 27-30 nm in diameter.

[0165] The kinetics of picornavirus replication is rapid, the cycle being completed in about 5-10 hours (typically by about 8 hours) (*see* Figure 68 for a schematic of the picornavirus replication cycle). Upon receptor binding, the genomic RNA is released from the particle into the cytoplasm. Genomic RNA is then translated directly by polysomes, but in about 30 minutes after infection, cellular protein synthesis declines sharply, almost to zero. This phenomenon is called "shutoff," and is a primary cause of cytopathic effects (cpe). Shutoff appears to be due to cleavage of the host cell's 220 kDa cap-binding complex (CBC) that is involved in binding the m7G cap structure at the 5' end of all eukaryotic mRNA during initiation of translation. The cleavage of the CBC appears to be caused by the 2A protease.

[0166] The 5' UTR contains the IRES. Normally, eukaryotic translation is initiated when ribosomes bind to the 5' methylated cap and then scans along the mRNA to find the first AUG initiation codon. The IRES overcomes this process and allows Picornavirus RNA's to continue to be translated after degradation of CBC.

[0167] The virus polyprotein is initially cleaved by the 2A protease into polyproteins P1, P2 and P3 (*see* Figure 4B). Further cleavage events are then carried out by 3C, the main picornavirus protease. One of the cleavage products made by 3C is the virus RNA-dependent RNA polymerase (3D), which copies the genomic RNA to produce a negative (-) sense strand. The (-) sense strand forms the template for the (+) strand (genomic) RNA synthesis. Some of the (+) strands are translated to produce additional viral proteins are some (+) strands are packaged into capsids to form new virus particles.

[0168] The (+) strand RNA genome is believed to be packaged into preformed capsids, although the molecular interactions between the genome and the capsid are not clear. Empty capsids are common in all picornavirus infections. The capsid is assembled by cleavage of the P1 polyprotein precursor into a protomer consisting of VP0, VP3, and VP1, which join together enclosing the genome. Maturation and infectivity of the virus particle relies on an internal autocatalytic cleavage of VP0 into VP2 and VP4. Release of newly formed virus particles occurs when the cell lyses.

[0169] The present invention also provides an isolated virus having all the identifying characteristics and nucleic acid sequence of ATCC Patent Deposit number PTA-5343. Viruses of the present invention can be directed to the PTA-5343 isolate, variants, homologues, derivatives and mutants of the PTA-5343 isolate, and variants, homologues, derivatives and mutants of other picornaviruses that are modified in respect to sequences of SVV (both wild-type as disclosed herein and mutant) that are determined to be responsible for its oncolytic properties.

[0170] The present invention further provides antibodies that are specific against: the isolated SVV having the ATTC Patent Deposit number PTA-5343, and epitopes from the isolated SVV proteins having the amino acid sequences SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22. The invention also includes antibodies that are specific against epitopes from the proteins that are encoded by fragments or portions of SEQ ID NO:1.

[0171] Comparative analyses of the RNA sequences from a variety of cardiovirus isolates have shown >45% nucleotide identity between genomes. Cardioviruses can be subclassified into the EMC-like viruses ("EMCV" - such as, Mengo, B, R; and also MM, ME, Columbia-SK), the Theiler's-like viruses ("TMEV" - such as, BeAn, DA and GD VII strains), and the Vilyuisk viruses.

[0172] In analyzing the SVV sequence to other viruses, it appears that SVV is a cardiovirus (see Example 4 and Figures referenced therein). If EMCV and TMEV are taken as the standard cardioviruses, SVV is clearly not a typical cardiovirus. However, even these two viruses have their differences, notably in the 5' UTR (Pevear et al., 1987, J. Virol., 61: 1507-1516). Phylogenetically SVV clusters with EMCV and TMEV in much of its polyprotein (P1, 2C, $3C^{pro}$ and $3D^{pol}$ regions; see Figures 31-37), indicating that SVV is most likely a cardiovirus.

Methods for Treating Cancer:

[0173] The present invention provides methods for cancer therapy using viruses modified in view of the oncolytic properties of SVV, including picornaviruses, derivatives, variants, mutants or homologues thereof. The present invention shows that wild-type SVV (*i.e.*, ATTC Patent Deposit number PTA-5343) has the ability to selectively kill some types of tumors. For example, SVV can

selectively kill tumor cells that have neurotropic or neuroendocrine properties, including small cell lung cancer (SCLC) and neuroblastomas. Other examples of neuroendocrine tumors that are contemplated to be treated by the viruses of the present invention include, but are not limited to: adrenal pheochromocytomas, gastrinomas (causing Zollinger-Ellison syndrome), glucagonomas, insulinomas, medullary carcinomas (including medullary thyroid carcinoma), multiple endocrine neoplasia syndromes, pancreatic endocrine tumors, paragangliomas, VIPomas (vasoactive intestinal polypeptide tumor), islet cell tumors, and pheochromocytoma.

[0174] Also encompassed in the present invention are the four types of neuroendocrine lung tumors. The most serious type, small cell lung cancer (SCLC), is among the most rapidly growing and spreading of all cancers. Large cell neuroendocrine carcinoma is a rare cancer that, with the exception of the size of the cells forming the cancer, is very similar to SCLC in its prognosis and in how patients are treated. Carcinoid tumors, also known as carcinoids, comprise the other 2 types of lung neuroendocrine cancer. These two types are typical carcinoid and atypical carcinoid.

[0175] Not being bound by theory, the ability of SVV to specifically kill tumor cells may include, but is not limited to: selective replication, apoptosis, lysis via tumor-selective cell entry, tumor-selective translation, tumor-selective proteolysis, tumor-selective RNA replication, and combinations thereof.

[0176] SVV has many advantageous characteristics over other oncolytic viruses, including modified adenoviruses, for example: (i) SVV has a very high selectivity for cancers with neural properties, including SCLC, Wilms' tumor, retinoblastoma, and neuroblastoma - for example, SVV shows a greater than 10,000-fold selectivity toward neuroendocrine tumor cells; (ii) SVV has been shown to have a 1,000 fold better cell-killing specificity than chemotherapy treatments; (iii) SVV exhibits no overt toxicity in mice following systemic administration with as high as 10^{14} viral particles per kilogram; (iv) the efficacy of SVV is very robust in that 100% of large pre-established tumors can be eradicated in mice, with no recurrence of tumor growth; (v) SVV can be purified to high titer and can be produced at more than 200,000 particles per cell in permissive cell lines; (vi) SVV has a small size (the SVV virus particle is less than 30 nm in diameter) enabling better penetration and spread in

tumors than other oncolytic viruses, (vii) SVV replicates quickly (less than 12 hours) and (viii) no modification of SVV is necessary for its use as a specific anti-tumor agent.

[0177] Further, initial studies (*see* Example 6) indicate some additional factors that make SVV an advantageous tool for oncolytic viral therapy: (i) human serum samples do not contain neutralizing antibodies directed against SVV; (ii) SVV is not inhibited by complement; and (iii) SVV is not inhibited by hemagglutination. All of these factors contribute to the fact that SVV exhibits a longer circulation time *in vivo* than other oncolytic viruses (for example, *see* Example 7).

[0178] The present invention provides methods for selectively killing a neoplastic cell in a cell population that comprises contacting an effective amount of SVV with said cell population under conditions where the virus can transduce the neoplastic cells in the cell population, replicate and kill the neoplastic cells. Besides methods where SVV kills tumor cells *in vivo*, the present methods encompass embodiments where the tumors can be: (1) cultured *in vitro* when infected by SVV; (2) cultured in the presence of non-tumor cells; and (3) the cells are mammalian (both tumor and non-tumor cells), including where the cells are human cells. The *in vitro* culturing of cells and infection by SVV can have various applications. For example, *in vitro* infection be used as a method to produce large amounts of SVV, as method for determining or detecting whether neoplastic cells are present in a cell population, or as a method for screening whether a mutant SVV can specifically target and kill various tumor cell or tissue types.

[0179] The present invention further provides an *ex vivo* method of treating cancer wherein cells are isolated from a human cancer patient, cultured *in vitro*, infected with a SVV which selectively kills the cancer cells, and the non-tumor cells are introduced back to the patient. Alternatively, cells isolated form a patient can be infected with SVV and immediately introduced back to the patient as a method for administering SVV to a patient. In one embodiment, the cancer cells are of a hematopoietic origin. Optionally, the patient may receive treatment (*e.g.*, chemotherapy or radiation) to destroy the patient's tumor cell *in vivo* before the cultured cells are introduced back to the patient. In one embodiment, the treatment may be used to destroy the patient's bone marrow cells.

[0180] SVV possesses potent antitumor activity against tumor cell-types with neural characteristics. SVV does not exhibit cytolytic activity against normal human, rat mouse, bovine or ovine cell lines or non-neural tumor cell lines. Further SVV is not cytotoxic to primary human hepatocytes. Table 1 below summarizes studies that have been conducted to determine the *in vitro* cytolytic potency of SVV against selected tumor cell types.

Table 1: SVV Cytolytic Potency Against Selected Tumor Cell-Types

Cell Line	Cell Type	EC_{50} (VP/cell)
H446	Human SCLC	0.0012
PER.C6	Human Embryonic Retinoblast	0.02
H69AR	SCLC-Multidrug Resistant	0.035
293	AD5 DNA Transformed Human Kidney	0.036
Y79	Human Retinoblastoma	0.00035
IMR32	Human Brain Neuroblastsoma	0.035
D283	Med Human Brain Cerebellar Medulloblastoma	0.25
SK-N-AS	Human Brain Neuroblastoma	0.474
N1E-115	Mouse Neuroblastoma	0.0028
BEKPCB3E1	Bovine embryonic Kidney cells transformed with Ad5E1	0.99
H1299	Human non-SCLC	7.66
ST	Porcine Testis	5.9
DMS153	Human SCLC	9.2
BEK	Bovine Embryonic Kidney	17.55
M059K	Human Brain Malignant Glioblastoma	1,061
PK15	Porcine Kidney	1,144
FBRC	Fetal Bovine Retina	10,170
HCN-1A	Human Brain	23,708
H460	Human LCLC	>30,000 (inactive)
Neuro 2A	Mouse Neuroblastoma	>30,000 (inactive)
DMS79	Human SCLC	>30,000 (inactive)
H69	Human SCLC	>30,000 (inactive)
C8D30	Mouse Brain	>30,000 (inactive)

Cell Line	Cell Type	EC_{50} (VP/cell)
MRC-5	Human Fetal Lung Fibroblast	>30,000 (inactive)
HMVEC	Neonatal vascular endothelial cells	>30,000 (inactive)
HMVEC	Adult vascular endothelial cells	>30,000 (inactive)
A375-S2	Human Melanoma	>30,000 (inactive)
SK-MEL-28	Melanoma	>30,000 (inactive)
PC3	Human prostate cancer	>30,000 (inactive)
PC3M2AC6	Human prostate cancer	>30,000 (inactive)
LnCap	Human Prostate cancer	>30,000 (inactive)
DU145	Human prostate cancer	>30,000 (inactive)

[0181] Murine studies (*see* Examples) show that SVV can specifically kill tumors with great efficacy and specificity *in vivo*. These *in vivo* studies show that SVV has a number of advantages over other oncolytic viruses. For example, one important factor affecting the ability of an oncolytic tumor virus to eradicate established tumors is viral penetration. In studies with adenoviral vectors, Ad5 based vectors had no effect on SCLC tumor development in athymic mice. Based on immunohistochemical results, adenovirus did not appear to penetrate the established tumors. In contrast, SVV was able to eliminate H446 SCLC tumors in athymic nude mice following a single systemic administration. SVV has a small size (<30 nm in diameter) enabling better penetration and spread in tumor tissue than other viruses, and thus, the small size of SVV may contribute to its ability to successfully penetrate and eradicate established tumors.

[0182] Chemoresistance is a major issue facing any patient that receives chemotherapy as a facet of cancer therapy. Patients that become chemoresistant often, if not always, have a much poorer prognosis and may be left with no alternative therapy. It is well known that one of the major causes of chemoresistance is the expression, over expression, or increased activity of a family of proteins called Multiple Drug Resistant proteins (MRPs). Applicants have found that a sensitivity of certain tumor cells for SVV is also correlated with the chemoresistant state of cancer cells and MRP expression. H69 is a chemosensitive (adriamycin) cell line that is resistant to SVV *in vitro*, whereas H69AR is a chemoresistant cell line that

overexpresses MRPs and is sensitive to SVV (*see* Table 1). Evidence indicates that overexpression of MRPs, including MDR, correlates with sensitivity of cells to SVV killing. Thus, in one embodiment, the present invention provides a method for treating cancer wherein SVV kills cells overexpressing an MRP.

[0183] The present invention also provides methods for treating diseases that are a result of abnormal cells, such as abnormally proliferative cells. The method comprises contacting said abnormal cells with SVV in a manner that results in the destruction of a portion or all of the abnormal cells. SVV can be used to treat a variety of diseases that are a result of abnormal cells. Examples of these diseases include, but are not limited to, cancers wherein the tumor cells display neuroendocrine features and neurofibromatosis.

[0184] Neuroendocrine tumors can be identified by a variety of methods. For example, neuroendocrine tumors produce and secrete a multitude of peptide hormones and amines. Some of these substances cause a specific clinical syndrome: carcinoid, Zollinger-Ellison, hyperglycemic, glucagonoma and WDHA syndrome. Specific markers for these syndromes are basal and/or stimulated levels of urinary 5-HIAA, serum or plasma gastrin, insulin, glucagon and vasoactive intestinal polypeptide, respectively. Some carcinoid tumors and about one third of endocrine pancreatic tumors do not present any clinical symptoms and are called 'nonfunctioning' tumors. Therefore, general tumor markers such as chromogranin A, pancreatic polypeptide, serum neuron-specific enolase and subunits of glycoprotein hormones have been used for screening purposes in patients without distinct clinical hormone-related symptoms. Among these general tumor markers chromogranin A, although its precise function is not yet established, has been shown to be a very sensitive and specific serum marker for various types of neuroendocrine tumors. This is because it may also be elevated in many cases of less well-differentiated tumors of neuroendocrine origin that do not secrete known hormones. At the moment, chromogranin A is considered the best general neuroendocrine serum or plasma marker available both for diagnosis and therapeutic evaluation and is increased in 50-100% of patients with various neuroendocrine tumors. Chromogranin A serum or plasma levels reflect tumor load, and it may be an independent marker of prognosis in patients with midgut carcinoids.

[0185] The present invention also provides a pharmaceutical composition comprising SVV and a pharmaceutically acceptable carrier. Such compositions, which can comprise an effective amount of SVV in a pharmaceutically acceptable carrier, are suitable for local or systemic administration to individuals in unit dosage forms, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or oral solutions or suspensions, oil in water or water in oil emulsions, and the like. Formulations for parenteral and non-parenteral drug delivery are known in the art. Compositions also include lyophilized and/or reconstituted forms of SVV. Acceptable pharmaceutical carriers are, for example, saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ), water, aqueous buffers, such as phosphate buffers and Tris buffers, or Polybrene (Sigma Chemical, St. Louis, MO) and phosphate-buffered saline and sucrose. The selection of a suitable pharmaceutical carrier is deemed to be apparent to those skilled in the art from the teachings contained herein. These solutions are sterile and generally free particulate matter other than SVV. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. Excipients that enhance infection of cells by SVV may be included.

[0186] SVV is administered to a host or subject in an amount that is effective to inhibit, prevent or destroy the growth of the tumor cells through replication of the virus in the tumor cells. Methods that utilize SVV for cancer therapy include systemic, regional or local delivery of the virus at safe, developable, and tolerable doses to elicit therapeutically useful destruction of tumor cells. Even following systemic administration, the therapeutic index for SVV is at least 10, preferably at least 100 or more preferably at least 1000. In general, SVV is administered in an amount of between $1x10^8$ and $1x10^{14}$ vp/kg. The exact dosage to be administered is dependent upon a variety of factors including the age, weight, and sex of the patient, and the size and severity of the tumor being treated. The viruses may be administered one or more times, which may be dependent upon the immune response potential of the host. Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. If necessary, the

immune response may be diminished by employing a variety of immunosuppressants, so as to permit repetitive administration and/or enhance replication by reducing the immune response to the viruses. Anti-neoplastic viral therapy of the present invention may be combined with other anti-neoplastic protocols. Delivery can be achieved in a variety of ways, employing liposomes, direct injection, catheters, topical application, inhalation, etc. Further, a DNA copy of the SVV genomic RNA, or portions thereof, can also be a method of delivery, where the DNA is subsequently transcribed by cells to produce SVV virus particles or particular SVV polypeptides.

[0187] A therapeutically effective dose refers to that amount of the virus that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of viruses can be determined by standard procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population of animals or cells; for viruses, the dose is in units of vp/kg) and the ED_{50} (the dose - vp/kg - therapeutically effective in 50% of the population of animals or cells) or the EC_{50} (the effective concentration - vp/cell (see Table 1 for example) - in 50% of the population of animals or cells). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50} or EC_{50}. Viruses which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of viruses lies preferably within a range of circulating concentrations that include the ED_{50} or EC_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilize

[0188] In yet another aspect, a method for treating a host organism having a neoplastic condition is provided, comprising administering a therapeutically effective amount of a viral composition of the invention to said host organism. In one embodiment, the neoplastic tissue is abnormally proliferating, and the neoplastic tissue can be malignant tumor tissue. Preferably, the virus is distributed throughout the tissue or tumor mass due to its capacity for selective replication in the tumor tissue. Neoplastic conditions potentially amenable to treatment with the methods of the invention include those with neurotropic properties.

Methods for Producing the Viruses of the Present Invention:

[0189] Methods for producing the present viruses to very high titers and yields are additional aspects of the invention. As stated, SVV can be purified to high titer and can be produced at more than 200,000 particles per cell in permissive cell lines. Cells that are capable of producing high quantities of viruses include, but are not limited to, PER.C6 (Fallaux *et al.*, Human Gene Therapy, 9:1909-1917, 1998), H446 (ATCC# HTB-171) and the other cell lines listed in Table 1 where the EC_{50} value is less than 10.

[0190] For example, the cultivation of picornaviruses can be conducted as follows. The virus of interest is plaque purified once and a well-isolated plaque is picked and amplified in a permissive cell line, such as PER.C6. A crude virus lysate (CVL) from the infected cells can be made by multiple cycles of freeze and thaw, and used to infect large numbers of permissive cells. The permissive cells can be grown in various tissue culture flasks, for example, 50x150 cm^2 flasks using various media, such as Dulbecco's modified Eagle medium (DMEM, Invitrogen, Carlsbad, CA)) containing 10% fetal bovine serum (Biowhitaker, Walkersvile, MD) and 10 mM magnesium chloride (Sigma, St Louis, MO). The infected cells can be harvested between 24-48 hours after infection or when complete cytopathic effects (CPE) are noticed, and are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellet is resuspended in the cell culture supernatant and is subjected to multiple cycles of freeze and thaw. The resulting CVL is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus can be purified by gradient centrifugation. For example, two rounds of CsCl gradients can suffice for SVV purification: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming 1A260 = 9.5 x 10^{12} particles (Scraba D.G., and Palmenberg, A.C. 1999. Cardioviruses (Picornaviridae). In:Encyclopedia of Virology, Second edition, R.G. Webster and A Granoff Eds). Titers of purified virus are also determined by a standard plaque assay using PER.C6 cells. The yield of SVV from PER.C6 cells are greater than 200, 000 particles per cell with particles to PFU ratio of about 100. The yields of SVV from other permissive cells (H446-ATCC# HTB-171) may be at least this high or higher.

[0191] In addition, several steps in a commercially attractive large scale Good Manufacturing Processes (GMP) are applicable to the purification of SVV. The invention also contemplates methods for purifying SVV that are based on methods for purifying adenoviruses. These methods include isolating SVV based on its density, since SVV has a very similar density to adenovirus and can be co-purified with adenovirus.

Methods for Detecting and Studying Tumors:

[0192] The present invention provides methods for detecting tumor or neoplastic cells in a patient using the viruses of the present invention. Cellular samples can be obtained from a patient and screened by incubating the sample with an epitope-tagged SVV (or other tumor-specific viruses provided by the invention, *i.e.*, tumor-specific mutant cardioviruses), and then screening the sample for bound SVV by detecting the epitope tag. Alternatively, the sample can be screened by detecting whether the SVV causes any cellular lysis. If SVV does cause cellular lysis, or if SVV can bind specifically to cells in the sample, this would indicate the possibility that the sample contains neoplastic or tumor cells known to be capable of being bound and/or infected by SVV.

[0193] Additionally, SVV can be used in a method for detecting a tumor cell *in vivo*. In such a method, epitope-tagged SVV can first be inactivated in a manner where SVV can still bind to tumor cells specifically but cannot replicate. Tumor cells that have bound SVV can be detected by assaying for the epitope tag. Detection of the epitope tag can be accomplished by antibodies that specifically bind the epitope, where the antibodies are either labeled (for example, fluorescently) or where the antibodies can then be detected by labeled secondary antibodies.

[0194] The present methods of detection encompass detecting any type of tumor or neoplastic cell that is specifically targeted by any virus of the present invention. Specific tumor types include, for example, neuroendocrine-type tumors, such as retinoblastomas, SCLC, neuroblastomas glioblastomas and medulloblastomas.

[0195] The present invention also provides the use of SVV as a tool to study tumor cells. SVV selectively destroys some tumor cell types, and has very little, if any, toxic effects on non-tumor cells. Because of these characteristics, SVV can be

used to study tumors and possibly discover a new tumor specific gene and/or pathway. In other words, there is some characteristic of the tumor cells that allows replication of SVV, wherein normal cells do not exhibit said characteristic. Upon identification of a new tumor specific gene and/or pathway, therapeutic antibodies or small molecules can then be designed or screened to determine whether these reagents are anti-tumor agents.

[0196] The present invention also provides a method for identifying all types of cancers that respond to SVV. In one embodiment, the method for identifying SVV-responsive cells comprises obtaining cells, contacting said cells with SVV and detecting cell killing or detecting viral replication. Cell killing can be detected using various methods known to one skilled in the art (*e.g.*, MTS assay, *see* High-Throughput section herein). Methods of detecting virus replication are also known to one skilled in the art (*e.g.*, observance of CPE, plaque assay, DNA quantification methods, FACS to detect quantity of virus in the tumor cells, RT-PCR assays to detect viral RNA, etc.). In one embodiment, the cells are cancer cells. Examples of cancer cells include, but are not limited to, established tumor cell lines and tumor cells obtained from a mammal. In one embodiment, the mammal is a human. In a further embodiment, the cells are cancer cells obtained from a human cancer patient.

[0197] The method for identifying SVV-responsive cancer cells may be used to discover tumor cell lines or tumor tissues that are permissive for SVV replication. Also, by determining the characteristics of permissive tumor cells, one may be able to identify characteristics of tumor cells that cause the cells to be selectively killed by SVV. The discovery of these characteristics could lead to new targets for cancer drugs. Also, the methods for identifying SVV responsive cancer cells could be used as a screen for human cancer patients who would benefit from treatment with SVV.

[0198] Since the natural host of SVV has not yet been determined, there is a need for an assay to detect SVV. Thus, the present invention provides methods of detecting SVV. In one embodiment, the detection assay is based on antibodies specific to SVV polypeptide epitopes. In another embodiment, the detection assay is based on the hybridization of nucleic acids. In one embodiment, RNA is isolated from SVV, labeled (*e.g.*, radioactive, chemiluminsecence, fluorescence, etc.) to make a probe. RNA is then isolated from test material, bound to nitrocellulose (or a similar

or functionally equivalent substrate), probed with the labeled SVV RNA, and the amount of bound probe detected. Also, the RNA of the virus may be directly or indirectly sequenced and a PCR assay developed based on the sequences. In one embodiment, the PCR assay is a real time PCR assay.

Methods for Making Viruses with Altered Tropism:

[0199] The present invention provides methods for constructing SVV mutants (or variants or derivatives) where these mutants have an altered cell-type tropism. Specifically, SVV mutants are selected for their ability to specifically bind and/or kill tumor or neoplastic cells that are known to be resistant to wild-type SVV binding.

[0200] The native or wild-type SVV has a simple genome and structure that allow the modification of the native virus to target other cancer indications. These new derivatives have an expanded tropism toward non-neural cancers and still maintain the high therapeutic index found in the native SVV. One possible means of targeting is the inclusion of tissue-specific peptides or ligands onto the virus.

[0201] To select cancer-targeting viral candidates, the present invention provides methods to construct and screen an oncolytic virus library with a genetic insertion that encodes a random peptide sequence in the capsid region of the native SVV. A random peptide library with a diversity of 10^8 is believed to be sufficient and should yield peptides that specifically direct the virus to tumor tissue.

[0202] Various studies have shown that tumor cells exhibit different characteristics from normal cells such as: (1) tumor cells have more permeable cell membranes; (2) tumors have specific stromal cell types such as angiogenic endothelial cells which have previously been shown to express cell type specific receptors; and (3) tumor cells differentially express certain receptors, antigens and extracellular matrix proteins (Arap, W. *et al.*, Nat. Med., 2002, 8(2): 121-127; Kolonin, M. *et al.*, Curr. Opin. Chem. Biol., 2001, 5(3): 308-313; St. Croix, B. *et al.*, Science, 2000, 289(5482): 1997-1202). These studies demonstrated that tumor and normal tissues are distinct at the molecular level and targeted drug delivery and treatment of cancer is feasible. Specifically, several peptides selected by homing to blood vessels in mouse models have been used for targeted delivery of cytotoxic drugs (Arap, W. *et al.*, Science, 1998, 279(5349): 377-380), pro-apoptotic peptides

(Ellerby, H.M. *et al.*, Nat. Med., 1999, 17(8): 768-774), metalloprotease inhibitor (Koivunen, E. *et al.*, Nat. Biotechnol, 1999, 17(8): 768-774), cytokine (Curnis, F. *et al.*, Nat. Biotechnol., 2000, 18(11): 1185-1190), fluorophores (Hong. F.D. and Clayman, G.L., Cancer Res., 2000, 60(23): 6551-6556) and genes (Trepel, M. *et al.*, Hum. Gene Ther., 2000, 11(14): 1971-1981). The tumor-targeting peptides have proven to increase the efficacy and lower the toxicity of the parental drugs.

[0203] A library of SVV derivatives can be generated by the insertion of a random peptide sequence into the capsid region of the virus. As shown in Figure 57, a vector is first generated that contains the SVV capsid region, *i.e.*, "pSVV capsid." This capsid vector can then be mutagenized, for example, by cutting the vector with a restriction enzyme that cuts DNA at random positions, *i.e.*, CviJI (a blunt cutter). The vector is cut at numerous positions, and DNA that has been cut only once by CviJI can be isolated by gel-purification (*see* Figure 57). This isolated population of DNA contains a plurality of species that have been cut in the capsid region at different locations. This population is then incubated with oligonucleotides and ligase, such that a percentage of the oligonucleotides will be ligated into the capsid region of the vector at a number of different positions. In this manner, a library of mutant SVV capsids can be generated.

[0204] The oligonucleotides that are inserted into the capsid encoding region can be either random oligonucleotides, non-random oligonucleotides (*i.e.*, the sequence of the oligonucleotide is pre-determined), or semi-random (*i.e.*, a portion of the oligonucleotide is pre-determined and a portion has a random sequence). The non-random aspect of the contemplated oligonucleotides can comprise an epitope-encoding region. Contemplated epitopes include, but are not limited to, c-myc - a 10 amino acid segment of the human protooncogene myc (EQKLISEEDL (SEQ ID NO: 35); HA - haemoglutinin protein from human influenza hemagglutinin protein (YPYDVPDYA (SEQ ID NO: 36)); and His_6 - a sequence encoding for six consecutive histidines.

[0205] The library of mutant capsid polynucleotides (for example, "pSVV capsid library" in Figure 57) can then be digested with restriction enzymes such that only the mutant capsid encoding region is excised. This mutant capsid encoding region is then ligated into a vector containing the full-length genomic sequence minus

the capsid encoding region (*see* Figure 58, for example). This ligation generates a vector having a full-length genomic sequence, where the population (or library) of vectors comprise a plurality of mutant capsids. In Figure 58, this library of SVV mutants comprising different capsids is denoted as "pSVVFL capsid." The pSVVFL capsid vector library is then linearized and reverse-transcribed in order to generate mutant SVV RNA (*see* Figure 59). The mutant SVV RNA is then transfected into a permissive cell line such that those SVV sequences that do not possess a dehabilitating mutation in its capsid are translated by the host cells to produce a plurality of mutant SVV particles. In Figure 59, the plurality of mutant SVV particles are denoted as a "SVV capsid library."

[0206] The peptide encoded by the oligonucleotide inserted into the capsid encoding region can serve as a targeting moiety for specific viral infection. The viruses that target a specific type of cancer would selectively infect only those cancer cells that have a receptor to the peptide, replicate in those cells, kill those cells, and spread to only those same types of cells. This methodology enables the identification of novel tumor-targeting peptides and ligands, tumor-selective receptors, therapeutic SVV derivatives and other virus derivatives, including picornavirus derivatives.

[0207] *In vitro* and *in vivo* screening of SVV mutant libraries have several advantages over other technologies such as peptide bead libraries and phage display. Unlike these other technologies, the desirable candidate here, *i.e.* an SVV derivative that selectively binds to a cancer cell, will replicate *in situ*. This replication-based library approach has numerous advantages over prior methods of discovering new cell binding moieties, such as phage display. First, the screening of a SVV library is based on replication. Only the desired viral derivatives can replicate in the target tissue, in this case certain cancer cells. The screening/selection process will yield very specific viral candidates that have both the targeting peptide moiety and may be a cancer therapeutic itself. On the contrary, phage display screens will only result in binding events and yields only the targeting peptide candidates. Thus, SVV library screening offers a much faster and selective approach. Second, during *in vitro* or *in vivo* phage display screening, phages recovered from the target cells have to be amplified in bacteria, while SVV derivatives can be directly recovered and purified from infected cells (or from the culture supernatant of lytically infected cells). Third, SVV has a

smaller genome that renders easier manipulability; thus it is possible to randomly insert the genetic information into the capsid region to ensure an optimized insertion. Therefore, construction and screening of the SVV library has a high possibility to produce highly effective viral derivatives. These derivatives are designed and screened to specifically infect cancers with non-neural properties.

[0208] The insertion of oligonucleotides into the capsid encoding region will result in the generation of some defective mutants. Mutants may be defective in the sense that the insertion of an oligonucleotide sequence can result in a stop codon, such that the viral polyprotein will not be produced. Also, mutants may be defective in the sense that the insertion of an oligonucleotide sequence may result in the alteration of the capsid structure such that capsid can no longer be assembled. To decrease the probability that the insertion of oligonucleotide sequences may result in stop codon or untenable capsid structure, random oligonucleotides can be designed such that they do not encode for stop codons or for certain amino acids using methods such as TRIM.

[0209] To determine whether there is an optimal insertion point in the capsid region for oligonucleotides, one can generate an RGD-SVV library (*see* Example 16). The polynucleotide encoding the SVV capsid is randomly cut, for example, with CviJI. The randomly linearized capsid polynucleotides are then ligated to oligonucleotides encoding at least the RGD amino acid sequence (arginine-glycine-aspartic acid). These RGD-capsid sequences are then ligated into SVV full-length sequence vectors that are missing the capsid sequence. RGD-SVV derivatives viruses are produced and tested for their ability to infect and replicate in certain integrin-expressing cell lines (as the RGD peptide has been shown to target entities to integrin receptors). The RGD-SVV derivatives that are successful in infecting the integrin-expressing cell lines are then analyzed to determine whether there is a predominant insertion site for the RGD oligonucleotide. This site can then be used for site-directed insertion of random, non-random or semi-random oligonucleotides.

[0210] Further, in comparing portions of the capsid encoding region between SVV and other picornaviruses (*see* Figure 28), there are various non-boxed regions between the viruses where the sequence similarity is at its lowest. These regions may be important in contributing to the different tropisms between the viruses. Thus, these

regions may be candidate locations for oligonucleotide insertion mutagenesis of the SVV capsid (and for other viral capsids).

Inactivated SVV as a Tumor-Specific Therapeutic:

[0211] Since SVV and SVV-capsid derivatives can target specific tumor cell-types and/or tissues, the SVV particle itself can be used as a delivery vehicle for therapeutics. In such a method, the need for the oncolytic abilities of SVV becomes optional, as the delivered therapeutic can kill the targeted tumor cell.

[0212] For example, the wild-type SVV can be inactivated such that the virus no longer lyses infected cells, but where the virus can still specifically bind and enter targeted tumor cell-types. There are many standard methods known in the art to inactivate the replicative functions of viruses. For example, whole virus vaccines are inactivated by formalin or β-propiolactone such that the viruses cannot replicate. The wild-type SVV may itself contain peptides that cause the apoptosis of cells. Alternatively, SVV can be irradiated. However, irradiated viruses should first be tested to ensure that they are still capable of specifically targeting tumor cells, as certain irradiation conditions may cause protein, and thus capsid, alterations. Further, mutant SVVs can be generated where the packaging signal sequence is deleted. These SVV mutants are able to specifically bind and enter target cells, but replicated SVV genomic RNA will not be packaged and assembled into capsids. However, this method may prove to be useful as initial entry of these mutant SVVs will cause host-protein synthesis shut-off such that tumor-cell death is still achieved.

[0213] Derivative SVVs having mutant capsids can also be inactivated and used to kill cancer cells. Derivative SVVs with oligonucleotides encoding epitope tags inserted into the capsid region can be used as vehicles to deliver toxins to tumor cells. As described herein, derivative SVVs can be randomly mutagenized and screened for tumor-specific tropisms. Toxins can be attached to the epitope tags, such that the virus delivers the toxin to tumor cells. Alternatively, therapeutic antibodies that specifically bind to the epitope tag can be used, such that the virus delivers the therapeutic antibody to the tumor cell.

High-Throughput Screening:

[0214] The present invention encompasses high-throughput methods for screening viruses that have the ability to specifically infect different cell-lines. The specificity of infection can be detected by assaying for cytopathic effects. For example, a number of different tumor cell-lines can be grown in different wells of a multi-well plate that is amenable for high-throughput screening, for example a 384 well-plate. To each well, a sample of virus is added to test whether the cells are killed by virus-mediated lysis. From those wells that show cytopathic effects, the media is collected such that any viruses in the media can be amplified by infecting permissive cell lines in flasks or large tissue culture plates. The viruses are grown such that the RNA can be isolated and the sequence analyzed to determine sequence mutations that may be responsible for providing a tumor cell-type specific tropism for a virus.

[0215] Various colorimetric and fluorometric methods can quickly assay cytopathic effects, including fluorescent-dye based assays, ATP-based assays, MTS assays and LDH assays. Fluorescent-dye based assays can include nucleic acid stains to detect dead-cell populations, as cell-impermeant nucleic acid stains can specifically detect dead-cell populations. If it is desired to simultaneously detect both live-cell and dead-cell populations, nucleic acid stains can be used in combination with intracellular esterase substrates, membrane-permeant nucleic acid stains, membrane potential-sensitive probes, organelle probes or other cell-permeant indicators to detect the live-cell population. For example, Invitrogen (Carlsbad, CA) offers various SYTOX™ nucleic acid stains that only penetrate cells with compromised plasma membranes. Ethidium bromide and propidium iodide can also be used to detect dead or dying cells. These stains are high-affinity nucleic acid stains that can be detected by any light-absorbance reader

[0216] For example, lysis can be based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. To detect the presence of LDH in cell culture supernatants, a substrate mixture can be added such that LDH will reduce the tetrazolium salt INT to formazan by a coupled enzymatic reaction. The formazan dye can then be detected by a light-absorbance reader. Alternatively, an MTS assay [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] using phenzine methosulfate (PMS) as the electron coupling reagent can also be used to detect

cytotoxicity. Promega (Madison, WI) offers a CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay kit where the solution reagent is added directly to culture wells, incubated for 1-4 hours and then absorbance is recorded at 490 nm. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture.

[0217] There are numerous high-throughput devices for reading light-absorbance. For example, SpectraMax Plus 384 Absorbance Platereader (Molecular Devices) can detect wavelengths from 190-1000 nm in 1 nm increments. The device can read 96-well microplates in 5 seconds and 384-well microplates in 16 seconds for ultra fast sample throughput.

[0218] Virus replication can also be assayed as an indication of successful infection, and such detection methods can be used in a high-throughput manner. For example, real-time RT-PCR methods can be used to detect the presence of virus transcripts in cell-culture supernatants. Upon reverse-transcription of viral RNA into cDNA, the cDNA can be amplified and detected by PCR with the use of double-stranded DNA-binding dyes (for example, SYBR® Green, Qiagen GmbH, Germany). The amount of PCR product can then be directly measured using a fluorimeter.

[0219] Viruses from the wells showing cytopathic effects are grown up and tested in further *in vitro* (re-testing of tumor and normal cell lines) and *in vivo* models (testing whether the virus can kill explanted tumors in mice).

Antibodies:

[0220] The present invention is also directed to antibodies that specifically bind to the viruses of the present invention, including the proteins of the viruses. Antibodies of the present invention include naturally occurring as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric antibodies, bifunctional antibodies and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains (Huse *et al.*, Science 246:1275-1281, 1989). These and other methods of making, for example, chimeric, humanized, CDR-

grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246, 1993; Ward *et al.*, Nature 341:544-546, 1989; Harlow and Lane, Antibodies: A laboratory manual, Cold Spring Harbor Laboratory Press, 1988); Hilyard *et al.*, Protein Engineering: A practical approach, IRL Press 1992; Borrabeck, Antibody Engineering, 2d ed., Oxford University Press 1995). Antibodies of the invention include intact molecules as well as fragments thereof, such as Fab, F(ab')2, and Fv which are capable of binding to an epitopic determinant present in a polypeptide of the present invention.

[0221] Where a peptide portion of a SVV polypeptide of the invention (*i.e.*, any peptide fragment from SEQ ID NO:2) or peptide portion of another viral polypeptide of the invention used as an immunogen for antibody generation is non-immunogenic, it can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH), or by expressing the peptide portion as a fusion protein. Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (for example, by Harlow and Lane, *supra*, 1988). Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art (see, for example, Green *et al.*, "Production of Polyclonal Antisera," in Immunochemical Protocols, Manson, ed., Humana Press 1992, pages 1-5; Coligan *et al.*, "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," in Curr. Protocols Immunol. (1992), section 2.4.1).

[0222] Monoclonal antibodies also can be obtained using methods that are well known and routine in the art (Kohler and Milstein, Nature 256:495, 1975; Coligan *et al.*, *supra*, 1992, sections 2.5.1-2.6.7; Harlow and Lane, *supra*, 1988). For example, spleen cells from a mouse immunized with a virus, viral polypeptide or fragment thereof, can be fused to an appropriate myeloma cell line to produce hybridoma cells. Cloned hybridoma cell lines can be screened using, for example, labeled SVV polypeptide to identify clones that secrete monoclonal antibodies having the appropriate specificity, and hybridomas expressing antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies. Polyclonal antibodies similarly can be isolated, for example, from serum of an immunized animal. Such antibodies, in addition to being useful for performing a

method of the invention, also are useful, for example, for preparing standardized kits. A recombinant phage that expresses, for example, a single chain antibody also provides an antibody that can used for preparing standardized kits. Monoclonal antibodies, for example, can be isolated and purified from hybridoma cultures by a variety of well established techniques, including, for example, affinity chromatography with Protein-A SEPHAROSE gel, size exclusion chromatography, and ion exchange chromatography (Barnes *et al.*, in Meth. Mol. Biol. 10:79-104, Humana Press 1992); Coligan *et al.*, *supra*, 1992, see sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3).

[0223] An antigen-binding fragment of an antibody can be prepared by proteolytic hydrolysis of a particular antibody, or by expression of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol-reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see, for example, Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647; Nisonhoff *et al.*, Arch. Biochem. Biophys. 89:230. 1960; Porter, Biochem. J. 73:119, 1959; Edelman *et al.*, Meth. Enzymol., 1:422 (Academic Press 1967); Coligan *et al.*, *supra*, 1992, see sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

[0224] Another example of an antigen binding fragment of an antibody is a peptide coding for a single complementarity determining region (CDR). CDR peptides can be obtained by constructing polynucleotides encoding the CDR of an antibody of interest. Such polynucleotides can be prepared, for example, using the polymerase chain reaction to synthesize a variable region encoded by RNA obtained from antibody-producing cells (for example, Larrick *et al.*, Methods: A Companion to Methods in Enzymology 2:106, 1991).

[0225] The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in

various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

[0226] There are many different labels and methods of labeling antibodies known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or alternatively to the antigen, or will be able to ascertain such, using routine experimentation.

[0227] As various changes can be made in the above methods and compositions without departing from the scope and spirit of the invention as described, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

EXAMPLES

[0228] The examples described below are provided to illustrate the present invention and are not included for the purpose of limiting the invention.

Example 1

Amplification and Purification of Virus

[0229] Cultivation of SVV in PER.C6 cells: SVV is plaque purified once and a well isolated plaque is picked and amplified in PER.C6 cells (Fallaux et al., 1998). A crude virus lysate (CVL) from SVV infected PER.C6 cells is made by three cycles of freeze and thaw and used to infect PER.C6 cells. PER.C6 cells are grown in 50 x 150 cm^2 T.C. flasks using Dulbecco's modified Eagle medium (DMEM, Invitrogen,

Carlsbad, CA, USA)) containing 10% fetal bovine serum (Biowhitaker, Walkersvile, MD, USA) and 10 mM magnesium chloride (Sigma, St Louis, MO, USA). The infected cells harvested 30 hr after infection when complete CPE is noticed and are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellet is resuspended in the cell culture supernatant (30 ml) and is subjected to three cycles of freeze and thaw. The resulting CVL is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus is purified by two rounds of CsCl gradients: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming $1A_{260} = 9.5 \times 10^{12}$ particles (Scraba D.G., and Palmenberg, A.C. 1999. Cardioviruses (Picornaviridae). In:Encyclopedia of Virology, Second edition, R.G. Webster and A Granoff Eds). Titers of purified virus are also determined by a standard plaque assay using PER.C6 cells. The yield of SVV from PER.C6 cells are greater than 200, 000 particles per cell with particles to PFU ratio of about 100. The yields of SVV from other permissive cells (H446-ATCC# HTB-171) may be at least this high or higher.

Example 2

Electron Microscopy

[0230] SVV is mounted onto formvar carbon-coated grids using the direct application method, stained with uranyl acetate, and examined in a transmission electron microscope. Representative micrographs of the virus are taken at high magnification. For the transmission electron microscope, ultra-thin sections of SVV-infected PER.C6 cells are cut from the embedded blocks, and the resulting sections are examined in the transmission electron microscope.

[0231] The purified SVV particles are spherical and about 27 nm in diameter, appearing singly or in small aggregates on the grid. A representative picture of SVV is shown in Figure 2. In some places, broken viral particles and empty capsids with stain penetration are also seen. Ultrastructural studies of infected PER.C6 cells revealed crystalline inclusions in the cytoplasm. A representative picture of PER.C6 cells infected with SVV is shown in Figure 3. The virus infected cells revealed a few large vesicular bodies (empty vesicles).

Example 3

Nucleic Acid Isolation of SVV

[0232] RNA Isolation: SVV genomic RNA was extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen). Isolation was performed according to the supplier's recommendations. Briefly, 250 µl of the purified SVV was mixed with 3 volumes TRIZOL and 240 µl of chloroform. The aqueous phase containing RNA was precipitated with 600 µl isopropanol. The RNA pellet was washed twice with 70% ethanol, dried and dissolved in DEPC-treated water. The quantity of RNA extracted was estimated by optical density measurements at 260 nm. An aliquot of RNA was resolved through a 1.25% denaturing agarose gel (Cambrex Bio Sciences Rockland Inc., Rockland, ME USA) and the band was visualized by ethidium bromide staining and photographed (Figure 4).

[0233] cDNA synthesis: cDNA of the SVV genome was synthesized by RT-PCR. Synthesis of cDNA was performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase, and random 14-mer oligonucleotide or oligo-dT. Fragments of the cDNA were amplified, cloned into plasmids and the clones are sequenced

Example 4

SVV Sequence Analysis:

[0234] The nucleotide sequence of SVV SEQ ID NO:1 was analyzed to determine its evolutionary relationship to other viruses. The translated product (SEQ ID NO:2) for this ORF was picornavirus-like and reached from the middle of VP2 to the termination codon at the end of the 3D polymerase and was 1890 amino acids in length (Fig. 5A-5E and 7A-7B). The 3' untranslated region (UTR), nucleotides 5671-5734, which follows the ORF is 64 nucleotides (nt) in length, including the termination codon and excluding the poly(A) tail of which 18 residues are provided (Fig. 5E).

[0235] Preliminary comparisons (not shown) of three partial genome segments of SVV had revealed that SVV was most closely related members of the genus *Cardiovirus* (family *Picornaviridae*). Therefore an alignment of the polyprotein sequences of SVV, encephalomyocarditis virus (EMCV; species *Encephalomyocarditis virus*, Theiler's murine encephalomyelitis virus (TMEV; species *Theilovirus*), Vilyuisk human encephalomyelitis virus (VHEV; species *Theilovirus*) and a rat TMEV-like agent (TLV; species *Theilovirus*) was constructed (Fig. 28). From this alignment, the SVV polyprotein processing was compared to the polyprotein processing of the most closely related members of the Cardiovirus genus. Cleavage sites between the individual polypeptides is demarcated by the "/" character in Fig. 28.

[0236] In picornaviruses, most polyprotein cleavages are carried out by one or more virus-encoded proteases, although in cardio-, aphtho-, erbo- and teschoviruses the cleavage between P1-2A and 2B is carried out by a poorly understood *cis*-acting mechanism related to the 2A sequence itself and critically involving the sequence "NPG/P", where "/" represents the break between the 2A and 2B polypeptides (Donnelly et al., 1997, J. Gen. Virol. 78: 13-21). One of the parechoviruses, Ljungan virus, has this sequence (NPGP) present upstream of a typical parechovirus 2A and is either an additional 2A or is the C-terminal end of the P1 capsid region. In all nine currently recognised picornavirus genera, $3C^{pro}$ carries out all but the *cis*-acting self-cleaving reactions (i.e. 2A cleaves at its N-terminus in entero- and rhinoviruses and L cleaves at its C-terminus in aphthoviruses and erboviruses). The post-assembly cleavage of the capsid polypeptide VP0 to VP4 and VP2 is not carried out by $3C^{pro}$, but by an unknown mechanism which may involve the virus RNA. The VP0 cleavage does not occur in parechoviruses and kobuviruses. The normal cardiovirus $3C^{pro}$ cleavage site has either a glutamine (Q) or glutamate (E) at the -1 position and glycine (G), serine (S), adenine (A) or asparagine (N) at the +1 position (Table 2). The cleavages of the SVV polyprotein conform to this pattern except for the VP3/VP1 site which is histidine (H)/serine (S) (Table 2); however, H/S is probably present as the cleavage site between 3A and $3B^{VPg}$ in at least one strain of equine rhinitis A virus (ERAV; genus *Aphthovirus*) (Wutz et al., 1996, J. Gen. Virol. 77:1719-1730).

Table 2. Cleavage sites of SVV and cardioviruses

Between		SVV	EMCV	TMEV	Rat TLV	VHEV
L	VP4	Not known	LQ/GN	PQ/GN	PQ/GN	PQ/GN
VP4	VP2	Not known	LA/DQ	LL/DQ LM/DQ	LL/DQ	LL/DE
VP2	VP3	EQ/GP	RQ/SP	AQ/SP	PQ/SP	PQ/SP
VP3	VP1	FH/ST	PQ/GV	PQ/GV PQ/GI PQ/GS	PQ/GV	PQ/GV
VP1	2A	KQ/KM	LE/SP	LE/NP	LQ/NP	LE/NP
2A	2B	NPG/P*	NPG/P*	NPG/P*	NPG/P*	Nk
2B	2C	MQ/GP	QQ/SP	PQ/GP	AQ/SP	Nk
2C	3A	LQ/SP	AQ/GP AQ/AP	AQ/SP	AQ/SP	Nk
3A	3B	SE/NA	EQ/GP	EQ/AA	EQ/AA	Nk
3B	3C	MQ/QP	IQ/GP VQ/GP	IQ/GG	IQ/GG	Nk
3C	3D	MQ/GL	PQ/GA	PQ/GA	PQ/GA	Nk

*, the break between 2A and 2B is not a cleavage event.

[0237] Primary cleavages (P1/P2 and P2/P3) of SVV: These primary cleavage events are predicted to occur in a similar fashion to cardio-, aphtho-, erbo- and teschoviruses, involving separation of P1-2A from 2B by a novel mechanism involving the sequence NPG/P and a traditional cleavage event by $3C^{pro}$ between 2BC and P3 (Table 2).

[0238] P1 cleavages: Cleavages within the SVV P1 capsid coding region were relatively easy to predict by alignment with sequence with EMCV and TMEV (Table 2).

[0239] P2 cleavages: The 2C protein is involved in RNA synthesis. The 2C polypeptide of SVV contains NTP-binding motifs GxxGxGKS/T (domain A) and hyhyhyxxD (in which hy is any hydrophobic residue; domain B) present in putative helicases and all picornavirus 2Cs (Fig. 29).

[0240] P3 cleavages: Prediction of the P3 cleavage sites was also relatively straightforward. Little is known about the function of the 3A polypeptide. However, all picornavirus 3A proteins contain a putative transmembrane alpha-helix. Primary sequence identity is low in this protein between SVV and cardioviruses (See Fig. 28 between positions 1612 to 1701).

[0241] The genome-linked polypeptide, VPg, which is encoded by the 3B region, shares few amino acids in common with the other cardioviruses, however, the third residue is a tyrosine, consistent with its linkage to the 5' end of the virus genome (Rothberg et al., 1978). See Fig. 28 between positions 1703 and 1724.

[0242] The three-dimensional structure of four picornavirus 3C cysteine proteases have been solved and the active-site residues identified (HAV, Allaire et al., 1994, Nature, 369: 72-76; Bergmann et al., 1997, J. Virol., 71: 2436-2448; PV-1, Mosimann et al., 1997, J. Mol. Biol., 273: 1032-1047; HRV-14, Matthews et al., 1994, Cell, 77: 761-771; and HRV-2, Matthews et al., 1999, Proc. Natl. Acad. Sci. USA, 96: 11000-11007). The cysteine bolded in Fig. 29 is the nucleophile, while the first bolded histidine is the general base and the specificity for glutamine residues is defined mainly by the second bolded histidine; all three residues are conserved in the SVV sequence (Fig. 29) and all other known picornaviruses (Fig. 28; for 3C sequence comparison see between positions 1726 and 1946).

[0243] The 3D polypeptide is the major component of the RNA-dependent RNA polymerase and SVV contains motifs conserved in picorna-like virus RNA-dependent RNA polymerases, i.e. KDEL/IR, PSG, YGDD and FLKR (Fig. 3; Fig. 28 between positions 1948 and 2410).

[0244] Myristoylation of the N-terminus of P1: In most picornaviruses the P1 precursor polypeptide is covalently bound by its N-terminal glycine residue (when present the N-terminal methionine is removed) to a molecule of myristic acid via an amide linkage (Chow et al., 1987, Nature, 327: 482-486). Consequently the cleavage products VP0 and VP4 which contain the P1 N-terminus are also myristoylated. This myristoylation is carried out by myristoyl transferase which recognises an eight amino acid signal beginning with glycine. In picornaviruses, a five residue consensus sequence motif, G-x-x-x-T/S, has been identified (Palmenberg, 1989, In Molecular Aspects of Picornavirus Infection and Detection, pp. 211-241, Ed. Semler & Ehrenfeld, Washington D.C., Amer. Soc. for Micro.). Parechoviruses (*Human parechovirus* and *Ljungan virus*) as well as not having a maturation cleavage of VP0 are apparently not myristoylated, however, there appears to be some type of molecule blocking the N-terminus of VP0 for these viruses.

Comparisons of the individual SVV polypeptides with the public sequence databases

[0245] Each of the SVV polypeptides (SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22) were compared to the public protein sequence databases using the FASTA online program at the European Bioinformatics Institute (EBI; http://www.ebi.ac.uk/). The results (best matches) of these comparisons are shown in Table 3. The capsid polypeptides (VP2, VP3 and VP1) taken as a whole, along with 2C, $3C^{pro}$ and $3D^{pol}$ are most closely related to members of the cardiovirus genus, however, the short predicted 2A sequence is closer to that of Ljungan virus (genus *Parechovirus*). A more detailed comparison of the SVV 2A nucleotide sequence with similar sequences is shown in Fig. 28 (see also Fig. 70 for 2A-like NPG/P protein comparison).

Table 3. Database matches of individual predicted polypeptides of Seneca Valley virus

SVV polypeptide	Length (aa)	% identity	% identity ungapped	aa overlap	Organism	Matched protein
L (Leader)	No data	-	-	-	-	-
VP4 (1A)	No data	-	-	-	-	-
VP2 (1B)	>142	42.857	44.037	112	TMEV WW	VP2
		~ 51	-	~80	EMCV BEL-2887A/91	VP2
VP3 (1C)	239	44.068	46.637	236	EMCV ATCC VR-129B	VP3
VP1 (1D)	259	31.086	36.404	267	EMCV M100/1/02	VP1
2A	14	71.429	71.429	14	Ljungan virus 174F	2A1
2B	128	39.286	41.509	56	*Ureaplasma urealyticum*	Multiple banded antigen
2C	322	38.602	40.190	329	EMCV PV21	2C
3A	90	37.838	41.791	74	*Chlorobium tepidum* TLS*	Enolase 2†
$3B^{VPg}$	22	No matches	-	-	-	-
$3C^{pro}$	211	37.089	38.537	213	EMCV-R	3C protease
$3D^{pol}$	462	58.009	58.515	462	EMCV-PV21	3D polymerase

* a photosynthetic, anaerobic, green-sulfur bacterium
† 2-phosphoglycerate dehydratase 2) (2-phospho-D-glycerate hydro-lyase 2

[0246] The significance of the matches of SVV 2B with *Ureaplasma urealyticum* multiple banded antigen or 3A with *Chlorobium tepidum* endolase 2 is not clear, however, these relationships maybe worthy of further investigation.

Phylogenetic comparison of SVV polypeptides with other picornaviruses

[0247] Those SVV polypeptides which could be aligned with the cardioviruses (VP2, VP3, VP1, 2C, 3C and 3D) were compared with the same proteins of representative members of each of the picornavirus species (Table 4). The programs BioEdit v5.0.9 (Hall, 1999, Nucl. Acids. Symp. Ser., 41: 95-98) and Clustal X v1.83 (Thompson et al., 1997, Nucl. Acids Res., 25:4876-4882) were used to make the alignments and to construct distance matrices and unrooted Neighbor-joining trees according to the algorithm of Saitou and Nei (Satiou and Nei, 1987, Mol. Biol. Evol., 4: 406-425). Confidence limits on branches were accessed by bootstrap resampling (1000 pseudo-replicates). The trees were drawn using TreeView 1.6.6 (Page, 1996) (Figs. 31 to 37). The distance matrices used to construct the trees used values corrected for multiple substitutions, while Figures 38-44 show the actual percentage amino acid identities. Table 4 shows the current classification of the family *Picornaviridae* and the representative virus sequences used in these comparisons.

Table 4. The taxonomic classification of the picornaviruses used in the comparisons with SVV.

Genus	Species	Representative virus	Abbrev.	Acc. No.
Enterovirus	*Poliovirus*	Poliovirus 1	PV-1	V01149
	Human enterovirus A	Coxsackievirus A16	CV-A16	U05876
	Human enterovirus B	Coxsackievirus B5	CV-B5	X67706
	Human enterovirus C	Coxsackievirus A21	CV-A21	D00538
	Human enterovirus D	Enterovirus 70	EV-70	D00820
	Simian enterovirus A	Simian enterovirus A1	SEV-A	AF201894
	Bovine enterovirus	Bovine enterovirus 1	BEV-1	D00214
	Porcine enterovirus B	Porcine enterovirus 9	PEV-9	AF363453
New genus?	Not yet designated	Simian virus 2*	SV2	AY064708
	Porcine enterovirus A	Porcine enterovirus 8*	PEV-8	AF406813
Rhinovirus	*Human rhinovirus A*	Human rhinovirus 2	HRV-2	X02316
	Human rhinovirus B	Human rhinovirus 14	HRV-14	K02121
Cardiovirus	*Encephalomyocarditis virus*	Encephalomyocarditis virus	EMCV	M81861
	Theilovirus	Theiler's murine encephalomyelitis virus	TMEV	M20562
Aphthovirus	*Foot-and-mouth disease virus*	Foot-and-mouth disease virus O	FMDV-O	X00871
	Equine rhinitis A virus	Equine rhinitis A virus	ERAV	X96870
Hepatovirus	*Hepatitis A virus*	Hepatitis A virus	HAV	M14707
	Avian encephalomyelitis-like viruses	Avian encephalomyelitis virus	AEV	AJ225173
Parechovirus	*Human parechovirus*	Human parechovirus 1	HPeV-1	L02971
	Ljungan virus	Ljungan virus	LV	AF327920
Kobuvirus	*Aichi virus*	Aichi virus	AiV	AB040749
	Bovine kobuvirus	Bovine kobuvirus	BKV	AB084788
Erbovirus	*Equine rhinitis B virus*	Equine rhinitis B virus 1	ERBV-1	X96871
Teschovirus	*Porcine teschovirus*	Porcine teschovirus 1	PTV-1	AJ011380

* the current taxonomic status of SV2 and PEV-8 places them in the enterovirus genus, however, it has been suggested that they may be reclassified in a new genus (Krumbholz et al., 2002; Oberste et al., 2003).

[0248] The trees of the individual capsid proteins (Figs. 31 to 33) are not all representative of the tree produced when the data from all tree polypeptides is combined (Fig. 34). This is probably the result of difficulties in aligning the capsid polypeptides, particularly when they are not full length as is the case for VP2 (Fig. 31). However, the P1, 2C, $3C^{pro}$ and $3D^{pol}$ trees are all in agreement and show that SVV clusters with EMCV and TMEV.

Seneca Valley virus as a member of the cardiovirus genus

[0249] Clearly the $3D^{pol}$ of SVV is related to the cardioviruses, almost as closely as EMCV and TMEV are to each other (Fig. 37; Fig. 44). In the other polypeptides which are generally considered as being relatively conserved in picornaviruses, 2C and 3C, SVV is also most closely related to the cardioviruses although it is not as closely related to EMCV and TMEV as they are to each other (Fig. 42 and Fig. 43, respectively). In the outer capsid proteins (taken as a whole), SVV is also most closely related to the cardioviruses and has approximately the same relationship as the two aphthovirus species, *Foot-and-mouth disease virus* and *Equine rhinitis A virus* (~33%). SVV diverges greatly from the cardioviruses in the 2B and 3A polypeptides and has no detectable relationship with any known picornavirus.

However, this is not without precedent; avian encephalomyelitis virus differs considerably from hepatitis A virus (HAV) in 2A, 2B and 3A (Marvil et al., 1999, J. Gen. Virol., 80:653-662) but is tentatively classified within the genus *Hepatovirus* along with HAV.

[0250] Seneca Valley virus is clearly not a typical cardiovirus if EMCV and TMEV are taken as the standard. However, even these two viruses have their differences, notably in the 5' UTR (Pevear et al., 1987, J. Gen. Virol., 61: 1507-1516). However, phylogenetically SVV clusters with EMCV and TMEV in much of its polyprotein (P1, 2C, $3C^{pro}$ and $3D^{pol}$ regions). Ultimately, the taxonomic position of SVV within the Picornaviridae will be decided by the Executive Committee (EC) of the International Committee for the Taxonomy of Viruses (ICTV) following recommendations by the *Picornaviridae* Study Group and supporting published material. There are two options: i) include SVV as a new species in the cardiovirus genus; or ii) assign SVV to a new genus. At this stage, and for purposes of the present invention, SVV is in the cardiovirus genus.

Example 4

SDS-PAGE and N-Terminal Sequence Analysis of SVV Capsid Proteins

[0251] Purified SVV is subjected to electrophoresis using NuPAGE pre-cast Bis-Tris polyacrylamide mini-gel electrophoresis system (Novex, San Diego, Ca, USA). One half of the gel is visualized by silver stain while the other half is used to prepare samples for amino acid sequencing of the N-termini of the capsid proteins. Prior to transfer of proteins to membrane, the gel is soaked in 10 mM CAPS buffer, pH 11, for 1 hour, and a PVDF membrane (Amersham) is wetted in methanol. Proteins are transferred to the PVDF membrane. After transfer, proteins are visualized by staining with Amido black for approximately 1 minute, and bands of interest are excised with a scalpel and air dried. The proteins can be subjected to automated N-terminal sequence determination by Edman degradation using a pulsed phase sequencer.

[0252] Three major structural proteins of the purified SVV are shown in Figure 45 (approximately 36 kDa, 31 kDa, and 27 kDa).

Example 5

Assay for Neutralization Antibodies to SVV in Human Serum Samples

[0253] Preexisting antibodies to particular viral vectors may limit the use of such vectors for systemic delivery applications such as for treatment of metastatic cancer, because preexisting antibodies may bind to systemically delivered vectors and neutralize them before the vectors have a chance to transduce the targeted tissue or organ. Therefore, it is desirable to ensure that humans do not carry neutralization antibodies to viral vectors selected for systemic delivery. To determine whether human sera samples contain SVV-specific neutralizing antibodies, neutralization assays are carried out using randomly collected human sera samples.

[0254] Tissue culture infective dose 50: One day before the experiment, 180 µl of PER.C6 cell suspension containing $1x10^4$ cells are plated in 96-well tissue culture dish. The crude virus lysate (CVL) of SVV is diluted in log steps from 10^{-0} to 10^{-11} in DMEM medium (Dulbecco's Modified Eagle's Medium) and 20 µl of each dilution is transferred to three wells of a Falcon 96-well tissue culture plate containing PER.C6 cells. The plates are incubated at 37°C in 5% CO_2 and read at 3 days for microscopic evidence of cytopathic effect (CPE), and the tissue culture infective dose 50 ($TCID_{50}$) is calculated.

[0255] Neutralization assay: First, 40 µl of medium is placed in all the wells and then 40 µl of heat-inactivated serum is added to the first well and mixed by pipeting, making a 1:4 dilution used for screening purposes. 40 µl is then transferred to the next well to perform a two-fold dilution of the serum samples. 40 µl of SVV virus, containing 100 $TCID_{50}$, is added to wells containing diluted serum samples. Plates are incubated at 37°C for 1 hour. 40 µl of the mix is taken and transferred to a plate containing PER.C6 cells ($1x10^4$ cells/160 µl/well). The plates are incubated at 37°C for 3 days. After this time, the cultures are read microscopically for CPE.

[0256] In a representative neutralization assay performed as described above, twenty-two human sera samples randomly collected from USA, Europe and Japan were examined for SVV specific neutralizing antibodies. The serum samples were serially diluted and mixed with a fixed amount of SVV containing 100 $TCID_{50}$. Serum-virus mixtures were then used to infect PER.C6 cells and incubated for 24

hours. Neutralizing antibody titer was determined as the reciprocal of the highest dilution of serum able to block CPE formation. In this experiment, no dilution of serum blocked CPE formation indicating that the human serum samples did not contain SVV neutralizing antibodies.

[0257] Further SVV infection of PER.C6 was not inhibited by incubation with human blood (*see* Example 6), indicating that SVV infection was not inhibited by complement or by hemagglutination. As a result, SVV exhibits a longer circulation time *in vivo* than other oncolytic viruses, which is a significant problem with the use of oncolytic adenoviruses.

Example 6

Binding of SVV to Human Erythrocytes and Hemagglutination

[0258] Various viral serotypes have been shown to cause *in vitro* hemagglutination of erythrocytes isolated from blood of various animal species. Hemagglutination or binding to erythrocytes may cause toxicity *in vivo* and may also affect *in vivo* biodistribution and the efficacy of a viral vector. Therefore, it is desirable to analyze the erythrocyte agglutination properties of a viral vector selected for systemic administration to treat metastatic cancers.

[0259] Hemagglutination assay: To determine whether SVV causes agglutination of human erythrocytes, hemagglutination assays are carried out in U-bottom 96-well plates. Purified SVV is serially diluted in 25 µl PBS (Phosphate Buffered Saline) in duplicates, and an equal volume of 1% erythrocyte suspension is added to each well. Blood samples used for isolation of erythrocytes are obtained from healthy individuals with heparin as an anticoagulant. Erythrocytes are prepared by washing the blood three times in cold PBS to remove the plasma and the white blood cells. After the last wash, erythrocytes are suspended in PBS to make a 1% (V/V) cell suspension. The virus and erythrocytes are gently mixed and the plates are incubated at room temperature for 1 hour and monitored for a hemagglutination pattern.

[0260] Whole blood inactivation assay: To rule out direct inactivation of SVV by blood components, aliquots of virus are incubated with heparinized human blood belonging to A, B, AB and O blood groups or PBS for 30 minutes or 1 hour at room

temperature prior to separation of plasma, after which PER.C6 cells are infected and titers are calculated.

[0261] In representative assays performed as described above, no hemagglutination of human erythrocytes of different blood groups (A, B, AB and O) was seen at any tested dilutions of SVV. A slight increase in the virus titer is noticed when SVV is mixed with blood human samples and incubated for 30 minutes and 1 hour, indicating that the virus is not inactivated by blood components but becomes more infectious under tested conditions.

Example 7

In vivo Clearance

[0262] Blood circulation time: To determine the blood circulation time and the amount of the virus in the tumor, H446 tumor bearing nude mice were treated with SVV at a dose of $1x10^{12}$ vp/kg by tail vein injection. The mice were bled at 0, 1, 3, 6, 24, 48, 72 hours and 7 days (189 hours) post-injection and the plasma was separated from the blood immediately after collection, diluted in infection medium, and used to infect PER.C6 cells. The injected mice were sacrificed at 6, 24, 48, 72 hours and 7 days post-injection and the tumors were collected. The tumors were cut into small sections and suspended in one ml of medium and subjected to three cycles of freeze and thaw to release the virus from the infected cells. Serial log dilutions of supernatants were made and assayed for titer on PER.C6 cells. SVV titers were expressed as pfu/ml. The tumor sections were also subjected to H&E staining and immunohistochemistry to detect the virus capsid proteins in the tumor.

[0263] The circulating levels of virus particles in the blood were determined based on the assumption that 7.3% of mouse body weight is blood. In representative assays performed as essentially as described above, within 6 hours of virus administration, the circulating levels of SVV reduced to zero particles and SVV was not detectable at later time points (Fig. 46A). In the tumor, SVV was detectable at 6 hours post-injection, after which the amount of the virus increased steadily by two logs (Fig. 46B). The virus was detectable in the tumor as late as 7 days postinjection (Fig. 46B). The tumor sections when subjected to immunohistochemistry, revealed

SVV proteins in the tumor cells (Figure 47, top panels). When stained by H&E, the tumor sections revealed several rounded tumor cells (Figure 47, bottom panels).

[0264] SVV also exhibits a substantially longer resident time in the blood compared to similar doses of i.v. adenovirus. Following a single i.v. dose, SVV remains present in the blood for up to 6 hours (Figure 46C; Figure 46C is a duplication of Figure 46A for comparison purposes to Figure 46D), whereas adenovirus is cleared from the blood in about an hour (Figure 46D).

Example 8

Tumor Cell Selectivity

[0265] *In vitro* cell killing activity of SVV: To determine the susceptibility of human, bovine, porcine, and mouse cells, normal and tumor cells were obtained from various sources and infected with SVV. All cell types were cultured in media and under the conditions recommended by the supplier. Primary human hepatocytes may be purchased from In Vitro Technologies (Baltimore, MD) and cultured in Hepatocye Culture Media (HCM™, BioWhittaker/Clonetics Inc., San Diego, CA).

[0266] *In vitro* cytopathic assay: To determine which types of cells are susceptible to SVV infection, monolayers of proliferating normal cells and tumor cells were infected with serial dilutions of purified SVV. The cells were monitored for CPE and compared with uninfected cells. Three days following infection, a MTS cytotoxic assay is performed and lethal dose-50 percent (LD_{50}) values in particles per cell are calculated. See Tables 5 and 6 below.

Table 5. Cell lines with EC_{50} values less than 100

Cell lines with EC50 < 1	EC50 number
H446 (human sclc)	0.001197
PERC6	0.01996
H69AR (sclc-multidrug resisitant)	0.03477
293 (human kidney transformed with ad5E1)	0.03615
Y79 (human retinoblastoma)	0.0003505
IMR32 (human brain; neuroblastoma)	0.03509
D283med (human brain; cerebellum; medulloblastoma)	0.2503
SK-N-AS (human brain; neuroblastoma)	0.474
N1E-115 (mouse neuroblastoma)	0.002846

Cell lines with EC50 < 1	**EC50 number**
SK-NEP-1 (kidney, wilms' tumor, pleural effusion, human)	0.03434
BEKPCB3E1 (bovine embryonic kidney cells transformed with ad5E1	0.99
Cell Lines with EC50 < 10 (1-10)	**EC50 number**
H1299 (human-non sclc)	7.656
ST (pig testes)	5.929
DMS 153 (human sclc)	9.233
Cell lines with EC50 < 100 (10-100)	**EC50 number**
BEK (bovine embryonic kidney)	17.55

Table 6. Cell lines with EC_{50} values more than 1000

M059K (human brain; malignant glioblastoma)	HUVEC (human vein endothelial cells)	CMT-64 (mouse-sclc)
KK (human glioblastoma)	HAEC (human aortic endothelial cells)	LLC-1 (mouse-LCLC))
U-118MG (human glioblatoma)	WI38 (human lung fibroblast)	RM-1 (mouse-prostate)
DMS 79 (human sclc)	MRC-5 (human lung fibroblast)	RM-2 (mouse-prostate)
H69 (human sclc)	IMR90 (human lung fibroblast)	RM-9 (mouse-prostate)
DMS 114 (human sclc)	HMVEC (human microvascular endothelial cells-adult)	MLTC-1 (mouse-testes)
DMS 53 (human sclc)	HMVEC (human microvascular endothelial cells-neonatal)	KLN-205 (mouse-sqcc)
H460 (human-LCLC)	HCN-1A (human brain)	CMT-93 (mouse-rectal)
A375-S2 (human melanoma)	HRCE (human renal cortical epithelial cells)	B16F0 (mouse melanoma)
SK-MEL-28 (human melanoma)		Neuro-2A (mouse neuroblastoma)
PC3 (human prostate)		C8D30 (mouse brain)
PC3M2AC6 (human prostate)		PK15 (pig-kidney)
LNCaP (human prostate)		FBRC (fetal bovine retina)
DU145 (human prostate)		MDBK (bovine kidney)
Hep3B (human liver carcinoma)		CSL 503 (sheep lung cells transformed with ad5E1)
Hep2G (human liver carcinoma)		OFRC (ovine fetal retina cells)
SW620 (human-colon)		
SW839 (human kidney)		
5637 (human bladder)		
HeLa S3		
S8		

[0267] The MTS assay was performed according to the manufacturer's instructions (CellTiter 96® AQ_{ueous} Assay by Promega, Madison, WI). The CellTiter 96® AQ_{ueous} Assay preferably uses the tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent, phenazine methosulfate (PMS). Contact-inhibited normal human cells evaluated in the study include: HUVEC (human umbilical vein endothelial cells), HAEC (human aortic endothelial cells, Clonetics/BioWhittaker # CC-2535), Wi38 (normal human embryo lung fibroblasts, ATCC # CCL-75), IMR90 (human normal lung fibroblasts, ATCC CCL-186), MRC-5 (human normal lung fibroblasts, ATCC, # CCL-171) and HRCE (human renal cortical epithelial cells, Clonetics/BioWhittaker # CC-2554).

[0268] SVV does not produce CPE in any of the above contact-inhibited normal cells. No virus-induced CPE was seen in the following human tumor cell lines: Hep3B (ATCC # HB-8064), HepG2 (human hepatocellular carcinoma, ATCC # HB-8065), LNCaP (human prostate carcinoma, ATCC # CRL-10995), PC3M-2AC6, SW620 (human colorectal adenocarcinoma, ATCC # CCL-227), SW 839 (human kidney adenocarcinoma, ATCC # HTB-49), 5637 (human urinary bladder carcinoma, ATCC # HTB-9), DMS-114 (small cell lung cancer, ATCC # CRL-2066), DMS 153 (human small cell lung cancer, ATCC # CRL-2064), A549 (human lung carcinoma, ATCC # CCL-185), HeLa S3 (human cervical adenocarcinoma, ATCC # CCL-2.2), NCI-H460 (human large cell lung cancer, ATCC # HTB-177), KK (glioblastoma), and U-118 MG (human glioblastoma, ATCC # HTB-15). Note - the cell lines in Table 6 with EC_{50} values greater than 1000 are most likely not permissive for SVV replication and/or virion production; although the possibility remains that SVV can bind and enter into these cells but CPE is not observed because SVV replication cannot occur inside the cell or that replication does occur but CPE is not observed because there is some other post-entry block (*i.e.*, no packaging of replicated SVV genomes into virions). However, considering the absence of CPE in these cell lines, these cell-lines, and potentially tumor-types thereof, are good candidates to test which cell and tumor-types are permissive or non-permissive for SVV replication. Although wild-type SVV is tumor-specific, and has been shown to target neuroendocrine tumors, including small cell lung cancer and neuroblastomas, there may be individual

patients that have types of etiologies such that SVV is not permissive in their form of neuroendocrine tumor. Therefore, the invention does contemplate the generation of SVV derivatives that can kill tumor cell-types isolated from individual patients where the tumors are non-permissive to the wild-type SVV, and the tumor-types isolated from these individuals can include, for example, glioblastoma, lymphoma, small cell lung cancer, large cell lung cancer, melanoma, prostate cancer, liver carcinoma, colon cancer, kidney cancer, colon cancer, bladder cancer, rectal cancer and squamous cell lung cancer.

[0269] SVV-mediated cytotoxicity on primary human hepatocytes (In Vitro Technologies) was determined by LDH release assay (CytoTox® 96 Non-Radioactive Cytotoxicity Assay, Promega, # G1780). Primary human hepatocytes plated in collagen coated 12-well plates were infected with SVV at 1, 10 and 100 and 1000 particles per cell (ppc). After 3 hours of infection, the infection medium was replaced with 2 ml of growth medium and incubated for 3 days in a CO_2 incubator. The cell associated lactate dehydrogenase (LDH) and LDH in the culture supernatant was measured separately. Percent cytotoxicity is determined as a ratio of LDH units in supernatant over maximal cellular LDH plus supernatant LDH.

$$\text{Percent cytotoxicity} = \frac{\text{LDH units in culture supernatant X 100}}{\text{Sum of LDH units in supernatant and cell lysate}}$$

The data shown in Figure 48 illustrates the absence of SVV mediated hepatoxicity at all tested multiplicity of infections.

Example 10

Virus Production Assay

[0270] To assess the replicative abilities of SVV, several selected contact-inhibited normal cells and actively dividing tumor cells were infected with SVV at one virus particle per cell (ppc). After 72 hours, cells and the medium were subjected to three freeze-thaw cycles and centrifuged to collect the supernatant. Serial log dilutions of supernatants were made and assayed for titer on PER.C6 cells. For each cell line, the efficiency of SVV replication was expressed as pfu/ml (Figure 49).

Example 10

Toxicity

[0271] The maximum tolerated dose (MTD) is defined as the dosage immediately preceding the dose at which animals (*e.g.* mice) demonstrate a dose limiting toxicity (DLT) after the treatment with SVV. DLT is defined as the dose at which the animals exhibit a loss in body weight, symptoms, and mortality attributed to SVV administration during the entire duration of the study. Neutralizing antibodies to SVV were assessed at baseline, day 15, and day 21. Neutralization assays were carried as described earlier.

[0272] Escalating doses ($1x10^8$ - $1x10^{14}$ vp/kg) of SVV were administered intravenously into both immune deficient nude and caesarean derived-1 (CD-1) out-bred immune competent mice purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) to determine the MTD with 10 mice per dose level. The virus was well-tolerated at all tested dose levels without exhibiting any clinical symptoms and without loss in body weight (Figure 50). Mice were bled at day 15 and 21 and the sera was monitored for the presence of SVV-specific neutralizing antibodies in neutralization assays. SVV injected CD1 mice develop neutralizing antibodies and the titers range from 1/1024 to greater than 1/4096.

[0273] Another toxicity study was conducted on the immunocompetent mouse strain (A/J). It has been demonstrated that SVV exhibits cell killing activity and replication in N1E-115 cells (see Table 1). The murine cell line N1E-115 (a neuroblastoma cell line, *i.e.*, neuroendocrine cancer) is derived from the A/J mouse strain. Thus, a syngeneic mouse model was established where N1E-115 cells were implanted subcutaneously in A/J mice to form tumors, and the mice were then treated with SVV to investigate its efficacy and toxicity.

[0274] In the A/J study, mice were i.v. injected with SVV to determine whether A/J mice can tolerate systemic administration of SVV. Blood hematology results were obtained to look for signs of toxicity, and serum chemistry results can also be obtained. The study design is shown in Table 7 below:

Table 7: A/J Study Design

Group #	Animals (Female)	Test Article	Dosage Level (particles/kg)	Dosage Volume (mL/kg)	Dosing regimen	Necropsy Day
1	5	Vehicle	0	10	IV on	Day 15

					Day 1	
2	5	SVV	10^8	10	IV on Day 1	Day 15
3	5	SVV	10^{11}	10	IV on Day 1	Day 15
4	5	SVV	10^{14}	10	IV on Day 1	Day 15

[0275] The A/J mice were 8-10 week old females obtained from The Jackson Laboratory (Bar Harbor, Maine). SVV was prepared by storing isolated virions at -80°C until use. SVV was prepared fresh by thawing on ice and diluting with HBSS (Hank's balanced salt solution). SVV was diluted to concentrations of 10^7 particles/mL for group 2, 10^{10} particles/mL for group 3, and 10^{13} particle/mL for group 4. HBSS was used as the vehicle control for group 1. All dosing solutions were kept on wet ice until dosing.

[0276] SVV was administered to animals intravenous injection via the tail vein at a dose volume of 10 mL/kg body weight. Animals were weighed on the day of dosing and dose volumes were adjusted based on body weight (*i.e.*, a 0.0200 kg mouse gets 0.200 mL of dosing solution). Mice were monitored twice daily for morbidity and mortality. Mice were weighed twice weekly. Information relating to moribund animals and animals exhibiting any unusual symptoms (physically or behaviorally) are recorded immediately.

[0277] Post-mortem observations and measurements entail the collection of blood from all surviving animals at terminal sacrifice for standard hematology and serum chemistry (AST, ALT, BUN, CK, LDH). The following organs are to be collected at sacrifice: brain, heart, lung, kidney, liver, and gonads. Half of each organ sample is snap frozen on dry ice and the other half will be placed in formalin.

[0278] Initial blood hematology results (CBC, differential) were obtained two weeks after SVV injection and the results are summarized below in Table 8 below. Five mice were tested from each test group (see Table 7):

Table 8: A/J Toxicity Results - Blood Hematology

	Test Group 1	**Test Group 2**	**Test Group 3**	**Test Group 4**
Body Weight Result ± SD (g): Day 0	21.48 ± 0.88	21.98 ± 1.93	22.58 ± 0.87	21.04 ± 1.67

Day 14	20.26 ± 0.93	20.92 ± 1.71	21.44 ± 0.84	21.26 ± 1.45
CBC Wet (Result ± SD (ref range)):				
White blood count (THSN/UL)	3.63 ± 1.57 (2.60-10.69)	4.5 ± 1.57 (2.60-10.69)	4.26 ± 0.94 (2.60-10.69)	4.72 ± 0.62 (2.60-10.69)
Red blood count (MILL/UL)	9.87 ± 0.03 (6.4-9.4)	9.49 ± 0.07 (6.4-9.4)	9.76 ± 0.37 (6.4-9.4)	9.71 ± 0.32 (6.4-9.4)
Hemoglobin (GM/DL)	15.37 ± 0.06 (11.5-16.1)	14.78 ± 0.29 (11.5-16.1)	15.12 ± 0.66 (11.5-16.1)	15.02 ± 0.63 (11.5-16.1)
Hematocrit (%)	46.03 ± 0.40 (36.1-49.5)	44.52 ± 0.49 (36.1-49.5)	45.7 ± 1.82 (36.1-49.5)	45.28 ± 1.69 (36.1-49.5)
MCV (FL)	46.67 ± 0.58 (45.4-60.3)	47.00 ± 0.0 (45.4-60.3)	47.0 ± 0.0 (45.4-60.3)	46.6 ± 0.55 (45.4-60.3)
MHC (PICO GM)	15.57 ± 0.06 (14.1-19.3)	15.70 ± 0.17 (14.1-19.3)	15.37 ± 0.06 (14.1-19.3)	15.43 ± 0.15 (14.1-19.3)
MCHC (%)	33.37 ± 0.12 (25.4-34.1)	33.14 ± 0.48 (25.4-34.1)	33.08 ± 0.22 (25.4-34.1)	33.14 ± 0.25 (25.4-34.1)
Platelet (THSN/UL)	885.33 ± 28.6 (592-2972)	758.2 ± 146.2 (592-2972)	874.8 ± 56.7 (592-2972)	897.2 ± 105.4 (592-2972)
Differential (Result ± SD (ref range)):				
Bands (THSN/UL)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)
Seg. Neutrophils (THSN/UL)	0.92 ± 0.27 (0.13-2.57)	1.16 ± 0.37 (0.13-2.57)	1.09 ± 0.38 (0.13-2.57)	0.96 ± 0.20 (0.13-2.57)
Lymphocytes (THSN/UL)	2.64 ± 1.26 (1.43-9.94)	2.98 ± 1.41 (1.43-9.94)	3.10 ± 0.56 (1.43-9.94)	3.70 ± 0.41 (1.43-9.94)
Monocytes (THSN/UL)	0.06 ± 0.04 (0.0-0.39)	0.15 ± 0.05 (0.0-0.39)	0.06 ± 0.03 (0.0-0.39)	0.05 ± 0.02 (0.0-0.39)
Eosinophils (THSN/UL)	0.01 ± 0.01 (0.0-0.24)	0.01 ± 0.01 (0.0-0.24)	0.01 ± 0.01 (0.0-0.24)	0.003 ± 0.01 (0.0-0.24)
Basophils (THSN/UL)	0.0 (0.0-0.0)	0.004 ± 0.005 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Atypical Lympho. (THSN/UL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Metamyelocytes (THSN/UL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Myelocytes (THSN/UL)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
NRBC (/100WBC)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Other (Result ± SD (ref range)):				
AST (SGOT) (U/L)	1762.8 ± 1129.8 (72-288)	899.0 ± 234.6 (72-288)	779.8 ± 312.2 (72-288)	843.2 ± 653.4 (72-288)
ALT (SGPT) (U/L)	2171.8 ± 2792.9	535.2 ± 272.8 (24-140)	555 ± 350.8 (24-140)	380.2 ± 385.7 (24-140)

	(24-140)			
BUN (MG/DL)	27.2 ± 0.8	24.8 ± 1.9	24.6 ± 5.5	28.2 ± 12.8
	(9-28)	(9-28)	(9-28)	(9-28)
Creatine phospho-	28312.8 ±	12194.4 ±	10157 ±	11829 ±
kinase (U/L)	20534.4	4049.2	5420.5	10363.9
	(0-800)	(0-800)	(0-800)	(0-800)
LDH (U/L)	6650.2 ±	3661.6 ±	3450.8 ±	2808.4 ±
	4788.6	933.6	972.6	1709.1
	(260-680)	(260-680)	(260-680)	(260-680)
Hemolytic Index	706.6 ± 423.4	477.6 ± 195.7	589.6 ±198.6	496.4 ± 321.1
(MG/DL HGB)	(0-70)	(0-70)	(0-70)	(0-70)

[0279] These results show that there are no abnormalities in blood hematology profiles obtained from mice treated with low, medium and high doses of SVV compared to blood hematology profiles obtained from untreated mice. From this study, it can be concluded that there are no measureable signs of toxicity following systemic administration of SVV, indicating that SVV is tolerated by A/J mice following i.v. injection.

Example 11

Efficacy

[0280] Athymic female nude mice (nu/nu) aged 6-7 weeks purchased from Harlan Sprague Dawley (Indianapolis, IN) were used in efficacy studies. Mice were injected subcutaneously with $5x10^6$ H446 cells into the right flank using manual restraint. Tumor sizes were measured regularly, and the volumes were calculated using the formula $\pi/6 \times W \times L^2$, where L= length and W = width of the tumor. When the tumors reach approximately 100-150 mm^3, mice (n=10) were randomly divided into groups. Mice were injected with escalating doses of SVV by tail vein injections at a dose volume of 10 ml/kg. A control group of mice was injected with an equivalent volume of HBSS. Dose escalation proceeds from $1x\ 10^7$ to $1x10^{13}$ particles per kilogram body weight. Antitumoral efficacy was determined by measuring tumor volumes twice weekly following SVV administration. Complete response was defined as complete disappearance of xenograft; partial response as regression of the tumor volume by equal to or more than 50%; and no response as continuous growth of tumor as in the control group.

[0281] Tumors from mice treated with HBSS grew rapidly and the tumor volumes reached more than 2000 mm^3 by study day 20 (Figure 51; *see* line with open diamond). In contrast, mice given one systemic injection of SVV at all tested doses (with the exception of the lowest dose) became tumor free by study day 20. In the lowest dose group, 8 mice became tumor free, one mouse had a very large tumor and the other had a small palpable tumor (25 mm^3) by study day 31. To evaluate the antitumor activity of SVV on large sized tumors, five mice from HBSS group bearing tumors >2000 mm^3 were systemically injected with a single dose of $1x10^{11}$ vp/kg on study day 20. For the duration of the follow-up period (11 days of after SVV injection), a dramatic regression of the tumor volumes were noted (Figure 51).

[0282] Figure 52 shows a picture of mice that were "untreated" with SVV (*i.e.,* treated with HBSS) or "treated" with SVV. As can be seen, the untreated mice had very large tumors and the treated mice showed no visible signs of tumor. Further, for unsacrified mice treated with SVV, no tumor regrowth was observed for the duration of the study, 200 days.

[0283] *In vitro* efficacy data for SVV for specific tumor cell lines is shown in Tables 1 and 5. The data shows that SVV specifically infects particular tumor cell types and does not infect normal adult cells, a significant advantage over any other known oncolytic virus. SVV has been shown to have 1,000 times better cell killing specificity than chemotherapy treatments (cell killing specifity values for SVV have been shown to be greater than 10,000, whereas cell killing specificity values for chemotherapy are around 10).

[0284] Specific cytotoxic activity of SVV was demonstrated in H446 human SCLC cells. Following a two-day incubation with increasing concentrations of SVV, cell viability was determined. The results are shown in Figure 53. Figure 53 shows cell survival following incubation of SVV with either H446 SCLC tumor cells (top graph) or normal human H460 cells (bottom graph). SVV specifically killed the tumor cells with an EC_{50} of approximately 10^{-3} particles per cell. In contrast, normal human cells were not killed at any concentration of SVV. Further, as summarized in Tables 1-3, SVV was also cytotoxic toward a number of other tumor cell lines, including SCLC-multidrug resistant tumor cells. The EC_{50} values for SVV cytotoxicity for the other tumor cell lines ranged from 10^{-3} to greater than 20,000

particles per cell. SVV was non-cytotoxic against a variety other non-neural tumors and normal human tissues. Additionally, SVV was not cytotoxic to primary human hepatocytes, as measured by LDH release at up to 1000 particles per cell (*see* Figure 48).

Example 12

Biodistribution and Pharmacokinetic Study in Rodents

[0285] Pharmacokinetic and biodistribution study of SVV is performed in normal mice and immunocompromised athymic nude mice bearing H446 SCLC tumors. This study evaluates the biodistribution, elimination and persistence of SVV following a single intravenous administration to both normal and immunocompromised tumor-bearing mice. Groups of mice each receive a single i.v. dose of control buffer or one of three doses of SVV (10^8, 10^{10}, or 10^{12} vp/kg) and are monitored for clinical signs. Blood samples are obtained from groups of 5 mice at 1, 6, 24 and 48 hours post dose, and at 1, 2, 4, and 12 weeks post dose. Dose levels include a known low efficacious dose and two higher dose levels to determine linearity of virus elimination. Groups of mice are sacrificed at 24 hours, and 2, 4 and 12 weeks post dose. Selected tissues, including liver, heart, lung, spleen, kidney, lymph nodes, bone marrow, brain and spinal cord tissues are aseptically collected and tested for the presence of SVV RNA using a validated RT-PCR assay.

[0286] Samples of urine and feces are obtained at sacrifice, at 24 hours, and at 2, 4 and 12 weeks post dose and are examined for the presence of infectious virus. The design of the experiments in this Example are shown in Table 9 below:

Table 9: Biodistribtuion of SVV in CD-1 Mice and Athymic Nude Mice Bearing SCLC Tumors

Group	Treatment	Dose Level (vp/kg)	Route	# of Mice/Timepoint for Blood Sampling	# of Mice/Timepoint for PCR Tissue Distribution
Normal CD-1 Mice					
1	Saline	0	i.v.	5	5
2	SVV	10^8	i.v.	5	5
3	SVV	10^{10}	i.v.	5	5
4	SVV	10^{12}	i.v.	5	5
Athymic Tumor Bearing Mice					
5	Saline	0	i.v.	5	5

6	SVV	10^8	i.v.	5	5
7	SVV	10^{10}	i.v.	5	5
8	SVV	10^{12}	i.v.	5	5

[0287] Acute i.v. toxicology studes were also performed in both normal and immunocompromised athymic nude mice bearing H446 SCLC tumors. Preliminary i.v. studies in normal and SCLC tumor bearing mice indicate safety of SVV at doses up to 10^{14} vp/kg. No adverse clinical signs were observed and there was no loss of body weight up to 2 weeks following a single i.v. dose of 10^{14} vp/kg.

Example 13

Viral Transmission Study in Normal Adult and Pregnant Mice

[0288] The purpose of this Example is to determine if SVV is transmissible following cohabitation of noninfected normal mice with mice injected with a high concentration of SVV. Because SVV does not replicate in normal, non-tumor bearing mice, tumor bearing mice can also be injected with high concentrations of SVV and subsequently exposed to normal, healthy animals to better simulate the clinical scenario. A secondary purpose is to assess the potential transmissibility of SVV from an infected female to an uninfected pregnant DAM, and subsequently to the developing fetus.

[0289] Three groups of five naive male and female CD-1 mice are exposed to a single mouse of the same sex infected with either 10^8, 10^{10} or 10^{12} vp/kg, and are monitored for the presence of SVV by blood sampling.

[0290] Similarly, an SVV exposed female is co-mingled with a number of timed pregnant females, and the ability of the virus to transmit from the infected female to an uninfected pregnant female, and subsequently to the developing fetus is determined.

Example 14

Non-Human Primate Studies

[0291] The safety, toxicity and toxicokinetics of SVV are also determined in non-human primates. In a dose range-finding phase, individual monkeys receive a single i.v. dose of SVV at 10^8 vp/kg and are closely monitored for clinical signs of infection or toxicity. If this dose is well tolerated, additional animals are treated with

a higher i.v. dose until a dose of 10^{12} vp/kg is achieved. Subsequently, the main study consists of groups of three male and female monkeys, and each monkey is dosed once weekly for six weeks with either vehicle alone or one of three doses of SVV and monitored for signs of toxicity. An additional two monkeys per sex are dosed with the vehicle alone and with the high dose level of SVV for six weeks, and are allowed an additional four weeks recovery prior to sacrifice.

[0292] Blood samples are obtained following dosing during week 1 and week 6. Clinical pathological and hematology blood samples are obtained prior to the initial dose and prior to sacrifice. Additional blood samples are obtained following each dose for assessing the presence of neutralizing antibodies to SVV.

[0293] Surviving monkeys are euthanized and subjected to a full gross necropsy and a full tissue list is collected from the main study and recovery monkeys. Tissues from the control and high dose groups are evaluated histopathologically. Urine and fecal samples are collected following dosing on weeks 1 and 6 and are evaluated for presence of infectious SVV. The overall design of this Example is shown in Table 10 below.

Table10: Multiple Dose Toxicology Study of SVV in Primates

Dose Range-finding Phase							
Group	Treatment	Dose (vp/kg)	Route	Males		Females	
1	SVV	10^{8}	IV	1		1	
2	SVV	10^{10*}	IV	1		1	
3	SVV	10^{12*}	IV	1		1	
Main Phase Recovery						**Main Phase**	
Group	Treatment	Dose (vp/kg)	Route	Male	Female	Male	Female
1	Control	-	IV	3	3	2	2
2	SVV	10^{8*}	IV	3	3	-	-
3	SVV	10^{10*}	IV	3	3	-	-
4	SVV	10^{12*}	IV	3	3	2	2

*Doses can vary based on results of Dose Rage-finding phase

Example 15

Construction of a Full-Length and Functional Genomic SVV Plasmid

[0294] To date, only about 1.5-2 Kb of the 5' genomic sequence of SVV remains to be sequenced, representing the nucleotide region covering the 5' UTR, 1A (VP4) and part of 1B (VP2). Additional SVV cDNAs are prepared from isolated SVV of ATCC deposit number PTA-5343. SVV particles are infected into a permissive cell line, such as PER.C6, and viruses are isolated. Viral RNA is then recovered from the virus particles such that cDNA copies are made therefrom. Individual cDNA clones are sequenced, such that selected cDNA clones are combined into one full-length clone in a plasmid having a T7 promoter upstream of the 5' end of the SVV sequence. The full-length SVV from this plasmid is reverse-transcribed, by utilizing T7 polymerase and an *in vitro* transcription system, in order to generate full-length RNA (*see* Figure 55). The full-length RNA is then transfected into permissive cell lines to test the infectivity of the full-length clone (*see* Figure 55).

[0295] The methodology is as follows. *RNA Isolation*: SVV genomic RNA is extracted using guanidium thiocyanate and a phenol extraction method using Trizol (Invitrogen). Briefly, 250 µl of the purified SVV is mixed with 3 volumes Trizol and 240 µl of chloroform. The aqueous phase containing RNA is precipitated with 600 µl isopropanol. The RNA pellet is washed twice with 70% ethanol, dried and dissolved in DEPC-treated water. The quantity of RNA extracted is estimated by optical density measurements at 260 nm. An aliquot of RNA is resolved through a 1.25% denaturing agarose gel (Cambrex Bio Sciences Rockland Inc., Rockland, ME USA) and the band visualized by ethidium bromide staining and photographed.

[0296] *cDNA synthesis:* cDNA of the SVV genome is synthesized by RT-PCR. Synthesis of cDNA is performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase, and random 14-mer oligonucleotide or oligo-dT. Fragments of the cDNA are amplified, cloned into the plasmid and the clones are sequenced. It is possible that more extensive measures are necessary to sequence the extreme 5' end of the genome.

[0297] *Cloning of full length genome:* Once the sequence is known routine molecular biology enables the construction of a full-length clone of SVV downstream of a T7 polymerase promoter (for example, *see* Figure 54).

[0298] *Recovery of SVV:* The plasmid with the full-length genome of SVV is reverse-transcribed following standard protocols. The viral RNA (100 ng) is used to transfect H446 cells, a cell line known to be permissive for the native SVV, but the most efficient cell line for viral RNA transfection can be empirically determined among a variety of cell lines.

Example 16

Construction of an RGD-Displaying SVV Library

[0299] To find the optimal insertion position for the construction of SVV capsid mutants generated with random with oligonucleotides encoding random peptide sequences, a simple model system (RGD) is employed. RGD (arginine, glycine, aspartic acid) is a short peptide ligand that binds to integrins. A successful RGD-SVV derivative should contain the following characteristics: (1) the genetic insertion should not alter any of SVV's unique and desirable properties; and (2) a successful RGD derivative virus should have tropism toward aVb5 integrin containing cells.

[0300] A SVV plasmid containing just the contiguous capsid region will be singly cleaved at random positions and a short model peptide sequence, referred to as RGD, will be inserted at each position. The virus SVV-RGD library will be constructed from this plasmid library utilizing the general technology described in Figures 56 and 57.

[0301] Random insertion of the cRGD oligonucleotide into the capsid region is conducted. In brief, a plasmid is constructed that just encodes the contiguous 2.1 Kb capsid region of SVV (*see* Figure 56, "pSVVcapsid"). A single random cleavage is made in pSVVcapsid by partially digesting the plasmid utilizing either CviJI or an endonuclease V method as described below (*see* Figure 57). After isolating the single cleaved plasmid the RGD oligonucleotide will be inserted to create a pSVVcapsid-RGD library.

[0302] The restriction enzyme CviJI has several advantages over other random cleavage methods such as sonication or shearing. First, as CviJI is a blunt ended cutter no repair is necessary. Second, CviJI has been demonstrated to cleave at random locations such that no hot spots will occur. The procedure is also simple and rapid. Briefly, the concentration of CviJI and/or time of digestion are increasingly

lowered until the majority of cleaved DNA is a linearized plasmid, *i.e.* a single cleavage. This can be observed by standard agarose gel electrophoresis as depicted in Figure 57. The band is then isolated, purified and ligated with the RGD oligo.

[0303] Another method that may be utilized to randomally cleave DNA is the endonuclease V method (Kiyazaki, K., Nucleic Acids Res., 2002, 30(24): e139). Endonuclease V nicks uracil-containing DNA at the second or third phosphodiester bond 3' to uracil sites. This method is also expected to randomly cleave DNA, the frequency is simply determined by adjusting the concentration of dUTP in the polymerase chain reaction. Although the cleavage sites are always two or three bases downstream of a thymidine (substituted by uracil) site, this method is expected to produce much fewer hot and cold spots than other methodologies.

[0304] The randomly linearized plasmids are ligated with the cRGD oligonucleotides. The resultant pSVV capsid library is then amplified, such that a population of polynucleotides encoding the capsid region with randomly inserted cRGD regions can be purified (*see* Figures 57 and 58). The population of capsid polynucleotides is then subcloned into a vector containing the full-length SVV sequence minus the capsid region, such that a library of full-length SVV sequences are generated (where the library manifests sequence diversity in the capsid region as the cRGD sequence is randomly inserted). This library is then reverse transcribed into RNA, and the RNA is transfected into a permissive cell line to generate a population of SVV particles having different capsids (*see* Figure 59). Once this RGD-SVV population of virus particles is recovered ("RGD-SVV library"), a number of viruses (*i.e.,* 10 or more) will be randomly picked for sequencing to confirm the insertion of the RGD sequence and diversity of insertion site.

[0305] *In vitro selection of the RGD-displaying SVV library.* The SVV-RGD library is screened to determine which insertion site enabled an expanded tropism of SVV. The RGD-SVV library is allowed to infect $\alpha_v\beta_5$ integrin-expressing NSCLC lines (non-small cell lung cancer cell lines, *i.e.,* A549 expressing $\alpha_v\beta_5$). Only those SVV derivatives that contain a functional and properly displayed RGD motif can infect these cells and replicate.

[0306] *In vitro* screening is carried out by a high throughput automation system (TECAN) that is capable of liquid handling, concurrent incubation of 20 cell lines and measurement in 384-well plates (*see* Figure 62 and Figure 63). The cells are harvested 30 hr after infection when complete CPE is noticed and then cells are collected by centrifugation at 1500 rpm for 10 minutes at 4°C. The cell pellets are then resuspended in the cell culture supernatant and subjected to three cycles of freeze and thaw. The resulting suspension is clarified by centrifugation at 1500 rpm for 10 minutes at 4°C. Virus is purified by two rounds of CsCl gradients: a one-step gradient (density of CsCl 1.24 g/ml and 1.4 g/ml) followed by one continuous gradient centrifugation (density of CsCl 1.33 g/ml). The purified virus concentration is determined spectrophotometrically, assuming $1A_{260} = 9.5 \times 10^{12}$ particles (Scraba, D.G. and Palmenberg, A.C., 1999). The process may be repeated multiple times until a sufficient amount of virus is recovered from $\alpha_V\beta_5$ cells.

[0307] *Analysis of recovered RGD-SVV derivatives.* A small pool of individual RGD-displaying SVV derivatives (about 10-50 different derivataives) are analyzed. The viral mixture is diluted and single viral particles are expanded for analysis. Each derivative is tested to determine whether they have gained the ability to infect $\alpha_V\beta_5$-expressing cells efficiently and specifically. The capsid region of each derivative with this property is then be sequenced to determine the site of RGD insertion. The recovered cRGD-displaying SVV derivatives should possess the following properties: (1) the original properties of the virus are still intact; and (2) the derivatives have gained the ability to infect cells that express high levels of integrins that bind to RGD. This approach aims to identify one or more sites that enable an expanded tropism with RGD insertion, such that random oligonucleotides can be inserted into these sites to generate SVV derivatives with altered tropism.

[0308] The sequenced cRGD-SVV derivatives are numbered and ranked by their binding abilities to integrin. To test the binding activity, recombinant β_2 integrin is immobilized on a 96-well microtiter plate in PBS, washed twice with PBS, blocked with 3% BSA in PBS, and then incubated with a unique RGD-displaying virus. The native virus without peptide insertions is used as a negative control. After 1-5 hr of incubation, the wells are washed at least three times with PBS. Then, the viruses that are bound to the plate will be detected by anti-SVV antibodies. RGD peptide or

antibodies against integrin should be able to compete with the binding of the RGD-SVV derivatives to the integrin-bound plate.

[0309] The cRGD-SVV derivatives (20) that have the strongest binding to integrin are analyzed to determine the 'successful' location(s) of cRGD oligonucleotide insertion. The insertion sites provide insights into the tropism of SVV. Based on the analysis of the insertion sites and other known structures, an ideal location to place a random peptide library can be determined (this method is an alternative method for generating SVV derivatives, where oligonucleotides (known sequence or random sequence) are inserted into random locations in the capsid). SVV derivatives generated with random sequence oligonucleotides are constructed in essentially the same manner as described above for the RGD-SVV library, except for two additional and novel methodologies. To avoid unwanted stop codons and deleterious amino acid insertions (e.g. cysteines or prolines) within a desired coding region, TRIM (trinucleotide-mutagenesis) technology developed by Morphosys (Munich, Germany) can be used to generate random oligonucleotides for capsid insertion. TRIM utilizes tri-nucleotides which only code for amino acids at the desired position (Virnekas, B. *et al.*, Nucleic Acids Res, 1994, 22(25): 5600-5607). The random-peptide displaying SVV with a diversity of 10^8 is believed to be sufficient and expected to yield peptides that specifically direct the virus to targeted tumor tissues. Random-peptide displaying SVV is tested *in vitro* as described above, or *in vivo* using tumor-bearing mice.

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid sequence having at least 95% sequence identity to SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or a contiguous portion of any one of these sequences that is at least 20 nucleotides in length.
2. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of high stringency.
3. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of moderate stringency.
4. An isolated nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of low stringency.
5. A vector comprising a nucleic acid sequence having at least 95% sequence identity to SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or a contiguous portion of any one of these sequences that is at least 20 nucleotides in length.
6. The isolated nucleic acid of claim 1, wherein the nucleic acid is RNA or DNA.
7. An isolated polypeptide encoded by a nucleic acid having at least 95% sequence identity to a nucleic acid sequence comprising SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or a contiguous portion of any one of these sequences that is at least 10 nucleotides in length.
8. An isolated polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or a contiguous portion of any one of these sequences that is at least 10 amino acids in length.
9. An isolated antibody which specifically binds to the polypeptide of claim 8 or to the isolated virus of claim 11.
10. The antibody of claim 9, wherein the antibody is a polyclonal antibody, a monoclonal antibody or a chimeric antibody.
11. An isolated Seneca Valley virus or derivative thereof, comprising identifying characteristics of ATCC Patent Deposit number PTA-5343.

12. An isolated Seneca Valley virus or derivative or relative thereof, having a genome comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70% or 65% identical to SEQ ID NO:1.

13. An isolated Seneca Valley virus or derivative or relative thereof, comprising the following characteristics: a single stranded RNA genome of about 7.5 kb; a diameter of ~27 nm; a capsid comprising at least 3 proteins that have approximate molecular weights of about 31 kDa, about 36 kDa, and about 27 kDa; a buoyant density of approximately 1.34 g/ml on CsCl gradients; and replication competence in tumor cells.

14. An isolated Seneca Valley virus or derivative or relative thereof, comprising the following characteristics: replication competence in tumor cells, tumor-cell tropism, and lack of cytolysis in normal cells.

15. The virus of claim 12 or 13, wherein said virus is replication competent in tumor cell types having neuroendocrine properties.

16. The virus of claim 12, wherein the 31 kDa protein comprises an amino acid sequence at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:8.

17. The virus of claim 12, wherein the 36 kDa protein comprises an amino acid sequence at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:4.

18. The virus of claim 12, wherein the 27 kDa protein comprises an amino acid sequence at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:6.

19. A pharmaceutical composition comprising an effective amount of the virus of any one of claims 11-18 and a pharmaceutically acceptable carrier.

20. A cell comprising the virus of any one of claims 11-18.

21. A viral lysate containing antigens of the virus of any one of claims 11-18.

22. An isolated viral antigen obtained from the virus of any one of claims 11-18.

23. A method for treating cancer comprising administering an effective amount of a virus or derivative thereof, so as to treat the cancer, wherein the virus has a genomic sequence that comprises a sequence that is at least 95% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21.

24. A method for treating cancer comprising administering an effective amount of a virus having a capsid encoding region comprising a sequence that is at least 95% identical to SEQ ID NO:3, 5 or 7.

25. A method for inhibiting cancer progression comprising contacting a cancer cell with a virus or derivative thereof, wherein the virus has a genome that comprises a sequence that is at least 95% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21.

26. A method for killing cancer cells comprising contacting a cancer cell with an effective amount of a virus or derivative thereof, wherein the virus has a genome that comprises a sequence that is at least 95% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 or 21.

27. The method of claim 23, 24, 25 or 26, wherein the virus is a picornavirus.

28. The method of claim 27, wherein the picornavirus is a cardiovirus.

29. The method of claim 28, wherein the cardiovirus is selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, encephalomyocarditis virus and Seneca Valley virus.

30. The method of claim 29, wherein the encephalomyocarditis virus is selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626.

31. The method of claim 29, wherein the Seneca Valley virus has an ATCC deposit number PTA-5343.

32. A method of purifying the virus of any one of claims 11-18, comprising:

    a. infecting a cell with the virus of any one of claims 11-18;

    b. harvesting cell lysate;

c. subjecting cell lysate to at least one round of gradient centrifugation; and

d. isolating said virus from the gradient.

33. A method of killing an abnormally proliferative cell comprising contacting the cell with the virus of any one of claims 11-18.

34. The method of claim 33, wherein the abnormally proliferative cell is a tumor cell.

35. The method of claim 34, wherein the tumor cell is selected from the group consisting of: human small cell lung cancer, human retinoblastoma, human neuroblastoma, human medulloblastoma, mouse neuroblastoma, wilms' tumor, and human non-small cell lung cancer.

36. A method of treating a neoplastic condition in a subject comprising administering to the subject an effective amount of the virus of any one of claims 11-18.

37. The method of claim 36, wherein the neoplastic condition is a neuroendocrine cancer.

38. The method of claim 36, wherein the subject is a human.

39. A method of producing the virus of any one of claims 11-18, comprising: culturing cells infected with the virus of any one of claims 11-18 under conditions that allow for replication of the virus and recovering the virus from the cells or the supernatant.

40. The method of claim 39, wherein the cells are PER.C6 cells.

41. The method of claim 39, wherein the cells are H446 cells.

42. The method of claim 39, wherein the cells produce over 200,000 virus particles/cell.

43. A method of detecting the virus of any one of claims 11-18, comprising: isolating RNA from test material suspected to contain the virus of any one of claims 11-18; labeling RNA corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1; probing the test material with the labeled RNA;

and detecting the binding of the labeled RNA with the RNA isolated from the test material, whereby binding indicates the presence of the virus.

44. A nucleic acid probe comprising a nucleotide sequence corresponding to at least 15 contiguous nucleotides of SEQ ID NO:1.

45. A method for making an oncolytic virus, the method comprising:

(a) comparing a Seneca Valley virus genomic sequence with a test virus genomic sequence;

(b) identifying at least a first amino acid difference between a polypeptide encoded by the Seneca Valley virus genomic sequence and a polypeptide encoded by the test virus genomic sequence;

(c) mutating the test virus genomic sequence such that the polypeptide encoded by the test virus genomic sequence has at least one less amino acid difference to the polypeptide encoded by the Seneca Valley virus genomic sequence;

(d) transfecting the mutated test virus genomic sequence into a tumor cell; and

(e) determining whether the tumor cell is lytically infected by the mutated test virus genomic sequence.

46. The method of claim 45, wherein the Seneca Valley virus genome comprises a sequence that is at least 95% identical to SEQ ID NO:1.

47. The method of claim 45, wherein the test virus is a picornavirus.

48. The method of claim 45, wherein the test virus is a cardiovirus.

49. The method of claim 45, wherein the amino acid differences are between a Seneca Valley virus capsid protein and a test virus capsid protein.

50. The method of claim 45, wherein mutating the test virus genomic sequence comprises mutating a cDNA having the test virus genomic sequence.

51. The method of claim 51, wherein transfecting the mutated test virus genomic sequence comprises transfecting RNA, wherein the RNA is generated from the cDNA having the mutated test virus genomic sequence.

52. The method of claim 48, wherein the cardiovirus genomic sequence is selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, and encephalomyocarditis virus.

53. The method of claim 52, wherein the cardiovirus genomic sequence is selected from an encephalomyocarditis virus.

54. The method of claim 53, wherein the encephalomyocarditis virus is selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626.

55. The method of claim 52, wherein the cardiovirus is selected from an isolate having a genome comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:1.

56. The method of claim 45, wherein the amino acid difference is within a polypeptide comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:4, 6, 8, or a contiguous portion of any one of these sequences that is at least 10 amino acids in length.

57. A method for making a mutant virus having an altered cell-type tropism, the method comprising:

(a) creating a library of viral mutants comprising a plurality of nucleic acid sequences;

(b) transfecting the library of viral mutants into a permissive cell, such that a plurality mutant viruses are produced;

(c) isolating the plurality of mutant viruses;

(d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and

(e) recovering a mutant virus that was produced in the non-permissive cell, thereby making a mutant virus having an altered tropism.

58. The method of claim 57, wherein the library of viral mutants is created from a parental sequence comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:1.

59. The method of claim 57, further comprising:

(f) incubating the recovered mutant virus in the non-permissive cell;

and

(g) recovering a mutant virus that that was produced in the non-permissive cell.

60. The method of claim 59, further comprising iteratively repeating steps (f) and (g).

61. The method of claim 57 or 59, wherein the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive cell-type in each well.

62. The method of claim 61, further comprising screening the platform to identify which wells contain a mutant virus that kills the cells.

63. The method of claim 62, wherein the screening is conducted by analyzing light absorbance in each well.

64. The method of claim 57, wherein the non-permissive cell is a tumor cell.

65. The method of claim 57, wherein creating the library of viral mutants comprises:

(i) providing a polynucleotide having a sequence identical to a portion of a genomic sequence of a virus;

(ii) mutating the polynucleotide in order to generate a plurality of different mutant polynucleotide sequences; and

(iii) ligating the plurality of mutated polynucleotides into a vector having the genomic sequence of the virus except for the portion of the genomic sequence of the virus that the polynucleotide in step (i) contains, thereby creating the library of viral mutants.

66. The method of claim 65, wherein the genomic sequence of a virus is from an picornavirus.

67. The method of claim 65, wherein the genomic sequence of a virus comprises a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70%, or 65% identical to SEQ ID NO:1.

68. The method of claim 66, wherein the picornavirus is a cardiovirus.

69. The method of claim 68, wherein the cardiovirus is selected from the group consisting of: vilyuisk human encephalomyelitis virus, Theiler's murine encephalomyelitis virus, encephalomyocarditis virus and SVV.

70. The method of claim 69, wherein the encephalomyocarditis virus is selected from the group of isolates consisting of: CA-131395, LA-97-1278, IL-92-48963, IA-89-47752, NJ-90-10324, MN-88-36695, and NC-88-23626.

71. The method of claim 57, wherein the mutating of step (ii) is conducted by random insertion of nucleotides into the polynucleotide.

72. The method of claim 57, wherein the mutating of step (ii) is conducted in a capsid encoding region of the polynucleotide.

73. The method of claim 71, wherein the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM).

74. The method of claim 71, wherein at least a portion of the nucleotides inserted into the polynucleotide encodes an epitope tag.

75. A method for making a mutant cardiovirus having an altered cell-type tropism, the method comprising:

(a) creating a library of mutant polynucleotide sequences of a cardiovirus, wherein the creating comprises:

- providing a polynucleotide encoding a capsid region of the cardiovirus;

- mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and

- ligating the plurality of mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the cardiovirus except for the capsid-encoding region, thereby creating the library of mutant polynucleotide sequences of the cardiovirus;

(b) transfecting the library of mutant polynucleotide sequences into a permissive cell, such that a plurality of mutant viruses is produced;

(c) isolating the plurality of mutant viruses;

(d) incubating a non-permissive cell with the isolated plurality of mutant viruses; and

(e) recovering a mutant virus that that was produced in the non-permissive cell, thereby making a mutant cardiovirus having an altered tropism.

76. The method of claim 75, further comprising:

(f) incubating the recovered mutant virus in the non-permissive cell; and

(g) recovering a mutant virus that that was produced in the non-permissive cell.

77. The method of claim 76, further comprising iteratively repeating steps (f) and (g).

78. The method of claim 75, wherein the cardiovirus has a genome that comprises a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70% or 65% identical to SEQ ID NO:1.

79. The method of claim 75, wherein the mutating is conducted by random insertion of nucleotides into the capsid-encoding polynucleotide.

80. The method of claim 80, wherein at least a portion of the nucleotides randomly inserted into the capsid-encoding polynucleotide encodes an epitope tag.

81. The method of claim 80, wherein the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM).

82. The method of claim 75, wherein the plurality of different mutant capsid-encoding polynucleotide sequences comprises greater than 10^8 different capsid-encoding polynucleotide sequences.

83. The method of claim 75, wherein the plurality of different mutant capsid-encoding polynucleotide sequences comprises greater than 10^9 different capsid-encoding polynucleotide sequences.

84. A method for making a mutant Seneca Valley virus having an altered cell-type tropism, the method comprising:

(a) creating a cDNA library of Seneca Valley virus mutants;

(b) generating Seneca Valley virus RNA from the cDNA library of

Seneca Valley virus mutants;

(c) transfecting the Seneca Valley virus RNA into a permissive cell, such that a plurality of mutant Seneca Valley viruses is produced;

(d) isolating the plurality of mutant Seneca Valley viruses;

(e) incubating a non-permissive tumor cell with the isolated plurality of mutant Seneca Valley viruses; and

(f) recovering a mutant Seneca Valley virus that lytically infects the non-permissive tumor cell, thereby making a mutant Seneca Valley virus having an altered tropism.

85. The method of claim 84, further comprising:

(g) incubating the recovered mutant Seneca Valley virus in the non-permissive cell; and

(h) recovering a mutant Seneca Valley virus that lytically infects the non-permissive tumor cell.

86. The method of claim 85, further comprising iteratively repeating steps (g) and (h).

87. The method of claim 85, wherein the incubating is conducted in a multi-well high-throughput platform wherein the platform comprises a different non-permissive tumor cell-type in each well.

88. The method of claim 87, further comprising screening the platform to identify which wells contain a mutant Seneca Valley virus that lytically infects the cells.

89. The method of claim 88, wherein the screening is conducted by analyzing light absorbance in each well.

90. The method of claim 84, wherein the cDNA library of Seneca Valley virus mutants comprises a plurality of mutant Seneca Valley virus capsid polynucleotide sequences.

91. The method of claim 90, wherein the plurality of mutant Seneca Valley virus capsid polynucleotide sequences is generated by random insertion of nucleotides into a capsid encoding region comprising a sequence that has at

least 95%, 90%, 85%, 80%, 75%, 70% or 65% sequence identity to SEQ ID NO:3, 5 or 7.

92. The method of claim 91, wherein at least a portion of the sequence of the nucleotides randomly inserted encodes an epitope tag.

93. The method of claim 91, wherein the random insertion of nucleotides is conducted by trinucleotide-mutagenesis (TRIM).

94. The method of claim 84, wherein the cDNA library of Seneca Valley virus mutants is generated from a Seneca Valley virus of ATCC deposit number PTA-5343 or a virus having a genome comprising a sequence that is at least 95%, 90%, 85%, 80%, 75%, 70% or 65% sequence identity to SEQ ID NO:1.

95. The method of claim 84, wherein the non-permissive tumor cell is a tumor cell-line or a tumor cell-type isolated from a patient.

96. The method of claim 95, wherein the non-permissive tumor cell-line is selected from the group consisting of: M059K, KK, U118MG, DMS79, H69, DMS114, DMS53, H460, A375-S2, SK-MEL-28, PC3, PC3M2AC6, LNCaP, DU145, Hep3B, Hep2G, SW620, SW839, 5637, HeLaS3, S8, HUVEC, HAEC, W138, MRC-5, IMR90, HMVEC, HCN-1A, HRCE, CMT-64, LLC-1, RM-1, RM-2, RM-9, MLTC-1, KLN-205, CMT-93, B16F0, Neuro-2A, C8D30, PK15, FBRC, MDBK, CSL503, and OFRC.

97. The method of claim 95, wherein the non-permissive tumor cell-type isolated from a patient is a selected from the group of cancers consisting of: glioblastoma, lymphoma, small cell lung cancer, large cell lung cancer, melanoma, prostate cancer, liver carcinoma, colon cancer, kidney cancer, colon cancer, bladder cancer, rectal cancer and squamous cell lung cancer.

98. A method for making a mutant virus having a tumor cell-type tropism *in vivo*, the method comprising:

(a) creating a library of viral mutants comprising a plurality of nucleic acid sequences;

(b) transfecting the library of viral mutants into a permissive cell, such that a plurality of mutant viruses are produced;

(c) isolating the plurality of mutant viruses;

(d) administering the isolated plurality of mutant viruses to a mammal with a tumor, wherein the mammal is not a natural host of the unmutated form of the mutant virus; and

(e) recovering a virus that replicated in the tumor, thereby making a mutant virus having a tumor cell-type tropism *in vivo*.

99. The method of claim 98, wherein the creating a library of viral mutants comprises:

- providing a polynucleotide encoding a capsid region of a virus;
- mutating the polynucleotide in order to generate a plurality of different mutant capsid-encoding polynucleotide sequences; and
- ligating the plurality of mutated capsid-encoding polynucleotides into a vector having the genomic sequence of the virus except for the capsid-encoding region, thereby creating the library of viral mutants.

100. The method of claim 98, wherein the virus recovered in step (e) lytically infects cells of the tumor.

101. The method of claim 98, wherein the tumor is a xenograft, a syngeneic tumor, an orthotopic tumor or a transgenic tumor.

102. The method of claim 98, wherein the mammal is a mouse.

103. The method of claim 98, wherein the viral mutant is a picornavirus.

104. The method of claim 98, wherein the picornavirus is a cardiovirus.

105. The method of claim 104, wherein the picornavirus is a Seneca Valley virus or a relative or a derivative thereof.

106. The method of claim 109, wherein the Seneca Valley virus is a Seneca Valley virus with the ATCC deposit number PTA-5343 or has a genomic sequence that comprises a sequence that is at least 95% identical to SEQ ID NO:1.

107. An oncolytic virus made by the method according to claim 45, 57, 75, 84 or 98.

108. A method for treating a patient with an oncolytic virus, the method comprising:

(a) inactivating an oncolytic virus of claim 107, such that the oncolytic virus is non-infectious and the tropism of the oncolytic virus is unaffected; and

(b) administering the inactivated oncolytic virus to a patient afflicted with a tumor.

109. The method of claim 108, further comprising attaching a toxin to the inactivated oncolytic virus.

110. A method for treating a patient with a tumor with Seneca Valley virus, the method comprising:

(a) inactivating a Seneca Valley virus such that the virus is non-infectious and the tropism of the virus is unaffected; and

(b) administering the inactivated Seneca Valley virus to a patient afflicted with a tumor.

111. The method of claim 110, further comprising attaching a toxin to the inactivated Seneca Valley virus.

112. A Seneca Valley virus composition comprising an inactivated Seneca Valley virus.

113. A Seneca Valley virus comprising an epitope tag incorporated in the capsid region.

114. A method for treating a subject with a tumor with Seneca Valley virus, the method comprising:

(a) creating a mutant Seneca Valley virus comprising an epitope tag encoded in the capsid;

(b) attaching a toxin to the epitope tag; and

(c) administering the mutant Seneca Valley virus with the attached toxin to a subject afflicted with a tumor.

115. The method of claim 114, wherein the creating comprises:

- inserting an oligonucleotide encoding an epitope tag into a capsid-encoding region polynucleotide of Seneca Valley virus.

116. The method of claim 115, wherein the mutant Seneca Valley virus does not have an altered cell-type tropism compared to a Seneca Valley virus of ATCC deposit number PTA-5343.

117. The method of claim 116, further comprising inactivating the mutant Seneca Valley virus such that the mutant Seneca Valley virus is not infectious.

118. A method for detecting a tumor cell in a sample comprising:

(a) isolating a tumor sample from a patient;

(b) incubating the tumor sample with an epitope-tagged Seneca Valley virus; and

(c) screening the tumor sample for bound Seneca Valley virus by detecting the epitope tag.

119. A method for detecting a tumor cell *in vivo* comprising:

(a) administering to a patient an irradiated epitope-tagged Seneca Valley virus, wherein a label is conjugated to the epitope-tag; and

(b) detecting the label in the patient.

120. The method of claim 118 or 119, wherein the Seneca Valley virus is a mutant, derivative or a relative.

121. A method for treating cancer with SVV comprising:

(a) making a SVV mutant comprising a deleted packaging signal sequence; and

(b) infecting a tumor cell with the SVV mutant, thereby causing the tumor cell death by SVV-mediated host-cell shutoff.

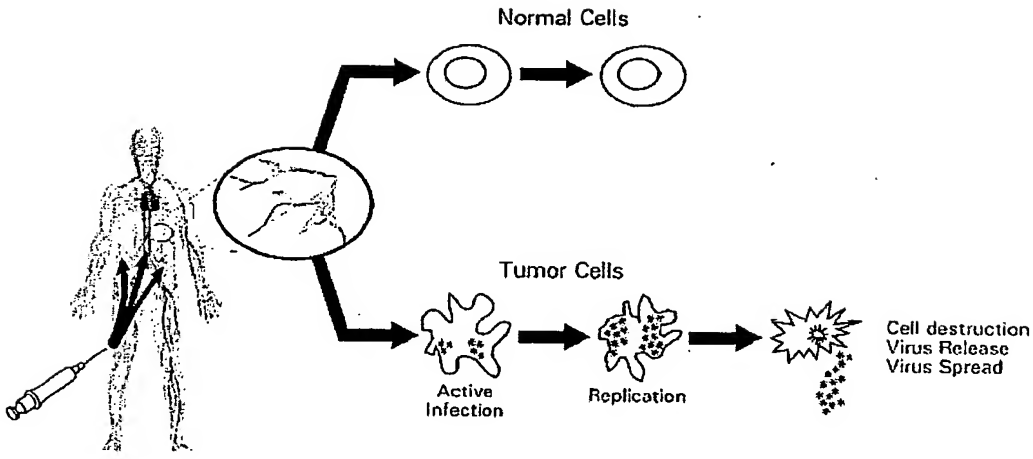
Normal Cells
Tumor Cells
Active Infection
Replication
Cell destruction
Virus Release
Virus Spread

FIG. 1

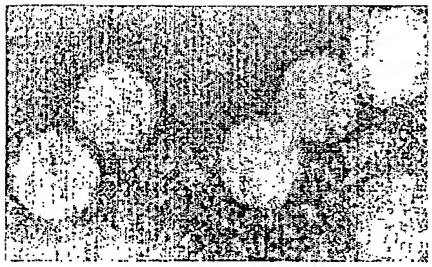

FIG. 2

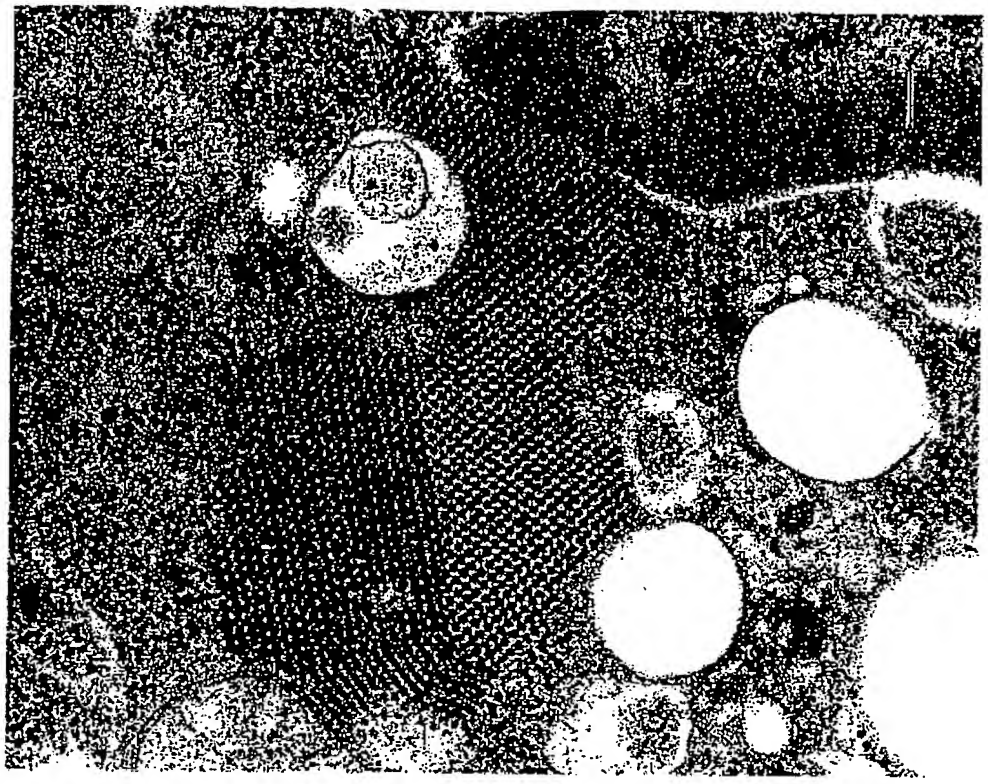

FIG. 3

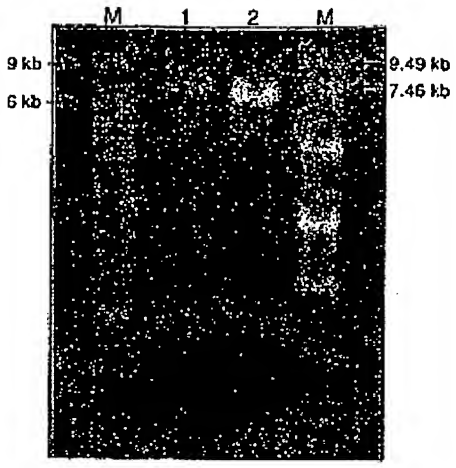

FIG. 4A

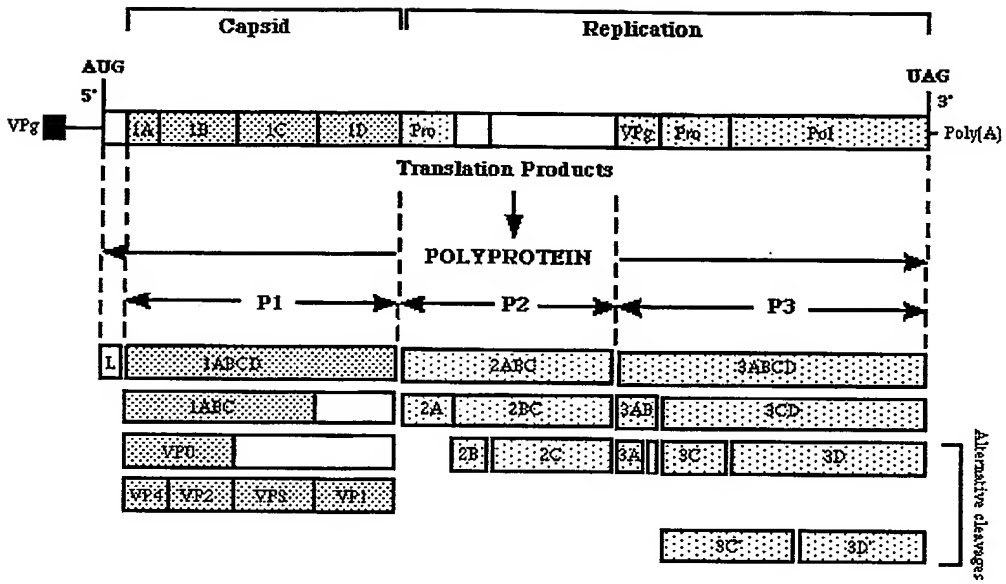
Capsid
Replication
AUG
5'
UAG
3'
VPg
1A
1B
1C
1D
Pro
VPg
Pro
Pol
Poly(A)
Translation Products
POLYPROTEIN
P1
P2
P3
L
1ABCD
2ABC
3ABCD
1ABC
2A
2BC
3AB
3CD
VP0
2B
2C
3A
3C
3D
VP4
VP2
VP3
VP1
3C
3D
Alternative cleavages

FIG. 4B

```
        10        20        30        40        50        60        70        80        90
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
NNTCTAGCCCACCATGGCAACAAGAAGAGCTTACAGGAGCTGAATGAAGAACAGTGGGTGGAAATGTCTGACGATTACCGGACCGGGAAA
 X  L  A  H  H  G  N  K  K  S  L  Q  E  L  N  E  E  Q  W  V  E  M  S  D  D  Y  R  T  G  K

       100       110       120       130       140       150       160       170       180
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AACATGCCTTTTCAGTCTCTTGGCACATACTATCGGCCCCCTAACTGGACTTGGGGTCCCAATTTCATCAACCCCTATCAAGTAACGGTT
 N  M  P  F  Q  S  L  G  T  Y  Y  R  P  P  N  W  T  W  G  P  N  F  I  N  P  Y  Q  V  T  V

       190       200       210       220       230       240       250       260       270
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTCCCACACCAAATTCTGAACGCGAGAACCTCTACCTCGGTAGACATAAACGTCCCATACATCGGGGAGACCCCCACGCAATCCTCAGAG
 F  P  H  Q  I  L  N  A  R  T  S  T  S  V  D  I  N  V  P  Y  I  G  E  T  P  T  Q  S  S  E

       280       290       300       310       320       330       340       350       360
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACACAGAACTCCTGGACCCTCCTCGTTATGGTGCTCGTTCCCCTAGACTATAAGGAAGGAGCCACAACTGACCCAGAAATTACATTTTCT
 T  Q  N  S  W  T  L  L  V  M  V  L  V  P  L  D  Y  K  E  G  A  T  T  D  P  E  I  T  F  S

       370       380       390       400       410       420       430       440       450
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTAAGGCCTACAAGTCCCTACTTCAATGGGCTTCGCAACCGCTACACGGCCGGGACGGACGAAGAACAGGGGCCCATTCCTACGGCACCC
 V  R  P  T  S  P  Y  F  N  G  L  R  N  R  Y  T  A  G  T  D  E  E  Q  G  P  I  P  T  A  P

       460       470       480       490       500       510       520       530       540
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGAGAAAATTCGCTTATGTTTCTCTCAACCCTCCCTGACGACACTGTCCCTGCTTACGGGAATGTGCGTACCCCTCCTGTCAATTACCTC
 R  E  N  S  L  M  F  L  S  T  L  P  D  D  T  V  P  A  Y  G  N  V  R  T  P  P  V  N  Y  L

       550       560       570       580       590       600       610       620       630
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCTGGTGAAATAACCGACCTTTTGCAACTGGCCCGCATACCCACTCTCATGGCATTTGAGCGGGTGCCTGAACCCGTGCCTGCCTCAGAC
 P  G  E  I  T  D  L  L  Q  L  A  R  I  P  T  L  M  A  F  E  R  V  P  E  P  V  P  A  S  D

       640       650       660       670       680       690       700       710       720
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACATATGTGCCCTACGTTGCCGTTCCCACCCAGTTCGATGACAGGCCTCTCATCTCCTTCCCGATCACCCTTTCAGATCCCGTCTATCAG
 T  Y  V  P  Y  V  A  V  P  T  Q  F  D  D  R  P  L  I  S  F  P  I  T  L  S  D  P  V  Y  Q

       730       740       750       760       770       780       790       800       810
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AACACCCTGGTTGGCGCCATCAGTTCAAATTTCGCCAATTACCGTGGGTGTATCCAAATCACTCTGACATTTTGTGGACCCATGATGGCG
 N  T  L  V  G  A  I  S  S  N  F  A  N  Y  R  G  C  I  Q  I  T  L  T  F  C  G  P  M  M  A

       820       830       840       850       860       870       880       890       900
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGAGGGAAATTCCTGCTCTCGTATTCTCCCCCAAATGGAACGCAACCACAGACTCTTTCCGAAGCTATGCAGTGCACATACTCTATTTGG
 R  G  K  F  L  L  S  Y  S  P  P  N  G  T  Q  P  Q  T  L  S  E  A  M  Q  C  T  Y  S  I  W

       910       920       930       940       950       960       970       980       990
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GACATAGGCTTGAACTCTAGTTGGACCTTCGTCGTCCCCTACATCTCGCCCAGTGACTACCGTGAAACTCGAGCCATTACCAACTCGGTT
 D  I  G  L  N  S  S  W  T  F  V  V  P  Y  I  S  P  S  D  Y  R  E  T  R  A  I  T  N  S  V
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FIG. 5A

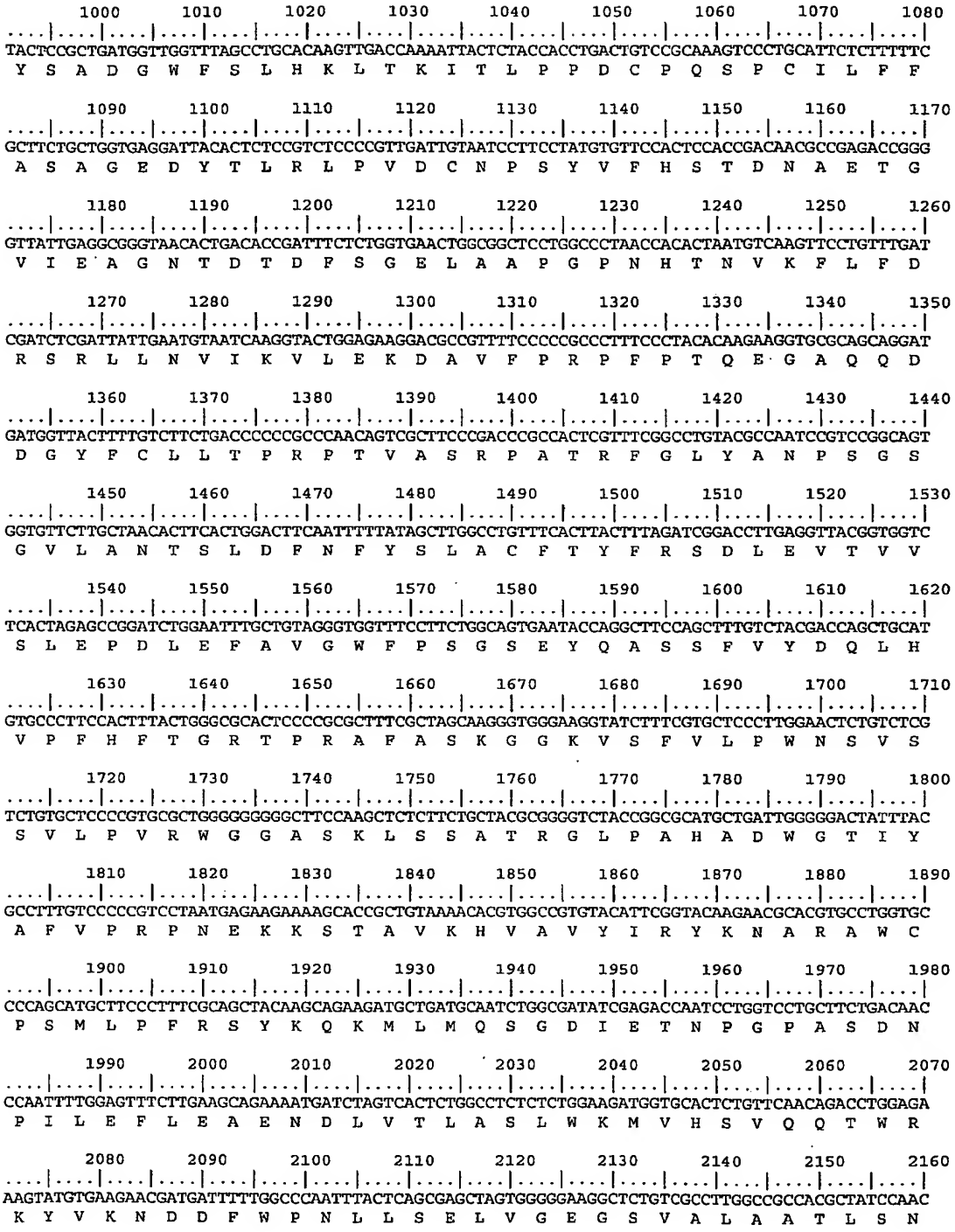
1000 1010 1020 1030 1040 1050 1060 1070 1080
TACTCCGCTGATGGTTGGTTTAGCCTGCACAAGTTGACCAAAATTACTCTACCACCTGACTGTCCGCAAAGTCCCTGCATTCTCTTTTTC
Y S A D G W F S L H K L T K I T L P P D C P Q S P C I L F F
1090 1100 1110 1120 1130 1140 1150 1160 1170
GCTTCTGCTGGTGAGGATTACACTCTCCGTCTCCCCGTTGATTGTAATCCTTCCTATGTGTTCCACTCCACCGACAACGCCGAGACCGGG
A S A G E D Y T L R L P V D C N P S Y V F H S T D N A E T G
1180 1190 1200 1210 1220 1230 1240 1250 1260
GTTATTGAGGCGGGTAACACTGACACCGATTTCTCTGGTGAACTGGCGGCTCCTGGCCCTAACCACACTAATGTCAAGTTCCTGTTTGAT
V I E A G N T D T D F S G E L A A P G P N H T N V K F L F D
1270 1280 1290 1300 1310 1320 1330 1340 1350
CGATCTCGATTATTGAATGTAATCAAGGTACTGGAGAAGGACGCCGTTTTCCCCCGCCCTTTCCCTACACAAGAAGGTGCGCAGCAGGAT
R S R L L N V I K V L E K D A V F P R P F P T Q E G A Q Q D
1360 1370 1380 1390 1400 1410 1420 1430 1440
GATGGTTACTTTTGTCTTCTGACCCCCCGCCCAACAGTCGCTTCCCGACCCGCCACTCGTTTCGGCCTGTACGCCAATCCGTCCGGCAGT
D G Y F C L L T P R P T V A S R P A T R F G L Y A N P S G S
1450 1460 1470 1480 1490 1500 1510 1520 1530
GGTGTTCTTGCTAACACTTCACTGGACTTCAATTTTTATAGCTTGGCCTGTTTCACTTACTTTAGATCGGACCTTGAGGTTACGGTGGTC
G V L A N T S L D F N F Y S L A C F T Y F R S D L E V T V V
1540 1550 1560 1570 1580 1590 1600 1610 1620
TCACTAGAGCCGGATCTGGAATTTGCTGTAGGGTGGTTTCCTTCTGGCAGTGAATACCAGGCTTCCAGCTTTGTCTACGACCAGCTGCAT
S L E P D L E F A V G W F P S G S E Y Q A S S F V Y D Q L H
1630 1640 1650 1660 1670 1680 1690 1700 1710
GTGCCCTTCCACTTTACTGGGCGCACTCCCCGCGCTTTCGCTAGCAAGGGTGGGAAGGTATCTTTCGTGCTCCCTTGGAACTCTGTCTCG
V P F H F T G R T P R A F A S K G G K V S F V L P W N S V S
1720 1730 1740 1750 1760 1770 1780 1790 1800
TCTGTGCTCCCCGTGCGCTGGGGGGGGGCTTCCAAGCTCTCTTCTGCTACGCGGGGTCTACCGGCGCATGCTGATTGGGGGACTATTTAC
S V L P V R W G G A S K L S S A T R G L P A H A D W G T I Y
1810 1820 1830 1840 1850 1860 1870 1880 1890
GCCTTTGTCCCCCGTCCTAATGAGAAGAAAAGCACCGCTGTAAAACACGTGGCCGTGTACATTCGGTACAAGAACGCACGTGCCTGGTGC
A F V P R P N E K K S T A V K H V A V Y I R Y K N A R A W C
1900 1910 1920 1930 1940 1950 1960 1970 1980
CCCAGCATGCTTCCCTTTCGCAGCTACAAGCAGAAGATGCTGATGCAATCTGGCGATATCGAGACCAATCCTGGTCCTGCTTCTGACAAC
P S M L P F R S Y K Q K M L M Q S G D I E T N P G P A S D N
1990 2000 2010 2020 2030 2040 2050 2060 2070
CCAATTTTGGAGTTTCTTGAAGCAGAAAATGATCTAGTCACTCTGGCCTCTCTCTGGAAGATGGTGCACTCTGTTCAACAGACCTGGAGA
P I L E F L E A E N D L V T L A S L W K M V H S V Q Q T W R
2080 2090 2100 2110 2120 2130 2140 2150 2160
AAGTATGTGAAGAACGATGATTTTTGGCCCAATTTACTCAGCGAGCTAGTGGGGGAAGGCTCTGTCGCCTTGGCCGCCACGCTATCCAAC
K Y V K N D D F W P N L L S E L V G E G S V A L A A T L S N

FIG. 5B

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        2170      2180      2190      2200      2210      2220      2230      2240      2250
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAAGCTTCAGTAAAGGCTCTTTTGGGCCTGCACTTTCTCTCTCGGGGGCTCAATTACACTGACTTTTACTCTTTACTGATAGAGAAATGC
 Q  A  S  V  K  A  L  L  G  L  H  F  L  S  R  G  L  N  Y  T  D  F  Y  S  L  L  I  E  K  C

        2260      2270      2280      2290      2300      2310      2320      2330      2340
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TCTAGTTTCTTTACCGTAGAACCACCTCCTCCACCAGCTGAAAACCTGATGACCAAGCCCTCAGTGAAGTCGAAATTCCGAAAACTGTTT
 S  S  F  F  T  V  E  P  P  P  P  P  A  E  N  L  M  T  K  P  S  V  K  S  K  F  R  K  L  F

        2350      2360      2370      2380      2390      2400      2410      2420      2430
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAGATGCAAGGACCCATGGACAAAGTCAAAGACTGGAACCAAATAGCTGCCGGCTTGAAGAATTTTCAATTTGTTCGTGACCTAGTCAAA
 K  M  Q  G  P  M  D  K  V  K  D  W  N  Q  I  A  A  G  L  K  N  F  Q  F  V  R  D  L  V  K

        2440      2450      2460      2470      2480      2490      2500      2510      2520
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAGGTGGTCGATTGGCTGCAGGCCTGGATCAACAAAGAGAAAGCCAGCCCTGTCCTCCAGTACCAGTTGGAGATGAAGAAGCTCGGGCCT
 E  V  V  D  W  L  Q  A  W  I  N  K  E  K  A  S  P  V  L  Q  Y  Q  L  E  M  K  K  L  G  P

        2530      2540      2550      2560      2570      2580      2590      2600      2610
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTGGCCTTGGCTCATGACGCTTTCATGGCTGGTTCCGGGCCCCCTCTTAGCGACGACCAGATTGAATACCTCCAGAACCTCAAATCTCTT
 V  A  L  A  H  D  A  F  M  A  G  S  G  P  P  L  S  D  D  Q  I  E  Y  L  Q  N  L  K  S  L

        2620      2630      2640      2650      2660      2670      2680      2690      2700
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GCCCTAACACTGGGGAAGACTAATTTGGCCCAAAGTCTCACCACTATGATCAATGCCAAACAAAGTTCAGCCCAACGAGTTGAACCCGTT
 A  L  T  L  G  K  T  N  L  A  Q  S  L  T  T  M  I  N  A  K  Q  S  S  A  Q  R  V  E  P  V

        2710      2720      2730      2740      2750      2760      2770      2780      2790
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTGGTGGTCCTTAGAGGCAAGCCGGGATGCGGCAAGGGCTTGGCCTCTACGTTGATTGCCCAGGCTGTGTCCAAGCGCCTCTATGGCTCC
 V  V  V  L  R  G  K  P  G  C  G  K  G  L  A  S  T  L  I  A  Q  A  V  S  K  R  L  Y  G  S

        2800      2810      2820      2830      2840      2850      2860      2870      2880
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CAAAGTGTATATTCTCTTCCCCCAGATCCAGATTTCTTCGATGGATACAAAGGACAGTTCGTGACCTTGATGGATGATTTGGGACAAAAC
 Q  S  V  Y  S  L  P  P  D  P  D  F  F  D  G  Y  K  G  Q  F  V  T  L  M  D  D  L  G  Q  N

        2890      2900      2910      2920      2930      2940      2950      2960      2970
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCGGATGGACAAGATTTCTCCACCTTTTGTCAGATGGTGTCGACCGCCCAATTTCTCCCCAACATGGCGGACCTTGCAGAGAAAGGGCGT
 P  D  G  Q  D  F  S  T  F  C  Q  M  V  S  T  A  Q  F  L  P  N  M  A  D  L  A  E  K  G  R

        2980      2990      3000      3010      3020      3030      3040      3050      3060
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCCTTTACCTCCAATCTCATCATTGCAACTACAAATCTCCCCCACTTCAGTCCTGTCACCATTGCTGATCCTTCTGCAGTCTCTCGCCGT
 P  F  T  S  N  L  I  I  A  T  T  N  L  P  H  F  S  P  V  T  I  A  D  P  S  A  V  S  R  R

        3070      3080      3090      3100      3110      3120      3130      3140      3150
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ATCAACTACGATCTGACTCTAGAAGTATCTGAGGCCTACAAGAAACACACACGGCTGAATTTTGACTTGGCTTTCAGGCGCACAGACGCC
 I  N  Y  D  L  T  L  E  V  S  E  A  Y  K  K  H  T  R  L  N  F  D  L  A  F  R  R  T  D  A

        3160      3170      3180      3190      3200      3210      3220      3230      3240
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCCCCCATTTATCCTTTTGCTGCCCATGTGCCCTTTGTGGACGTAGCTGTGCGCTTCAAAAATGGTCACCAGAATTTTAATCTCCTAGAG
 P  P  I  Y  P  F  A  A  H  V  P  F  V  D  V  A  V  R  F  K  N  G  H  Q  N  F  N  L  L  E

        3250      3260      3270      3280      3290      3300      3310      3320      3330
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTGGTCGATTCCATTTGTACAGACATTCGAGCCAAGCAACAAGGTGCCCGAAACATGCAGACTCTGGTTCTACAGAGCCCCAACGAGAAT
 L  V  D  S  I  C  T  D  I  R  A  K  Q  Q  G  A  R  N  M  Q  T  L  V  L  Q  S  P  N  E  N
```

FIG. 5C

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       3340      3350      3360      3370      3380      3390      3400      3410      3420
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GATGACACCCCCGTCGACGAGGCGTTGGGTAGAGTTCTCTCCCCCGCTGCGGTCGATGAGGCGCTTGTCGACCTCACTCCAGAGGCCGAC
 D  D  T  P  V  D  E  A  L  G  R  V  L  S  P  A  A  V  D  E  A  L  V  D  L  T  P  E  A  D

       3430      3440      3450      3460      3470      3480      3490      3500      3510
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCGGTTGGCCGTTTGGCTATTCTTGCCAAGCTAGGTCTTGCCCTAGCTGCGGTCACCCCTGGTCTGATAATCTTGGCAGTGGGACTCTAC
 P  V  G  R  L  A  I  L  A  K  L  G  L  A  L  A  A  V  T  P  G  L  I  I  L  A  V  G  L  Y


       3520      3530      3540      3550      3560      3570      3580      3590      3600
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGGTACTTCTCTGGCTCTGATGCAGACCAAGAAGAAACAGAAAGTGAGGGATCTGTCAAGGCACCCAGGAGCGAAAATGCTTATGACGGC
 R  Y  F  S  G  S  D  A  D  Q  E  E  T  E  S  E  G  S  V  K  A  P  R  S  E  N  A  Y  D  G

       3610      3620      3630      3640      3650      3660      3670      3680      3690
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCGAAGAAAAACTCTAAGCCCCCTGGAGCACTCTCTCTCATGGAAATGCAACAGCCCAACGTGGACATGGGCTTTGAGGCTGCGGTCGCT
 P  K  K  N  S  K  P  P  G  A  L  S  L  M  E  M  Q  Q  P  N  V  D  M  G  F  E  A  A  V  A

       3700      3710      3720      3730      3740      3750      3760      3770      3780
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AAGAAAGTGGTCGTCCCCATTACCTTCATGGTTCCCAACAGACCTTCTGGGCTTACACAGTCCGCTCTTCTGGTGACCGGCCGGACCTTC
 K  K  V  V  V  P  I  T  F  M  V  P  N  R  P  S  G  L  T  Q  S  A  L  L  V  T  G  R  T  F

       3790      3800      3810      3820      3830      3840      3850      3860      3870
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTAATCAATGAACATACATGGTCCAATCCCTCCTGGACCAGCTTCACAATCCGCGGTGAGGTACACACTCGTGATGAGCCCTTCCAAACG
 L  I  N  E  H  T  W  S  N  P  S  W  T  S  F  T  I  R  G  E  V  H  T  R  D  E  P  F  Q  T

       3880      3890      3900      3910      3920      3930      3940      3950      3960
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GTTCATTTCACTCACCACGGTATTCCCACAGATCTGATGATGGTACGTCTCGGACCGGGCAATTCTTTCCCTAACAATCTAGACAAGTTT
 V  H  F  T  H  H  G  I  P  T  D  L  M  M  V  R  L  G  P  G  N  S  F  P  N  N  L  D  K  F

       3970      3980      3990      4000      4010      4020      4030      4040      4050
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGACTTGACCAGATGCCGGCACGCAACTCCCGTGTGGTTGGCGTTTCGTCCAGTTACGGAAAGTTCTTCTTCTCTGGAAATTTCCTCGGA
 G  L  D  Q  M  P  A  R  N  S  R  V  V  G  V  S  S  S  Y  G  N  F  F  F  S  G  N  F  L  G

       4060      4070      4080      4090      4100      4110      4120      4130      4140
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTTGTTGATTCCGTCACCTCTGAACAAGGAACTTACGCAAGACTCTTTAGGTACAGGGTGACGACCTACAAAGGATGGTGCGGCTCGGCC
 F  V  D  S  V  T  S  E  Q  G  T  Y  A  R  L  F  R  Y  R  V  T  T  Y  K  G  W  C  G  S  A

       4150      4160      4170      4180      4190      4200      4210      4220      4230
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTGGTCTGTGAGGCCGGTGGCGTCCGACGCATCATTGGCCTGCATTCTGCTGGCGCCGCCGGTATCGGCGCCGGGACCTATATCTCAAAA
 L  V  C  E  A  G  G  V  R  R  I  I  G  L  H  S  A  G  A  A  G  I  G  A  G  T  Y  I  S  K

       4240      4250      4260      4270      4280      4290      4300      4310      4320
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTAGGACTAATCAAAGCCCTGAAACACCTCGGTGAACCTTTGGCCACAATGCAAGGACTGATGACTGAATTAGAGCCTGGAATCACCGTA
 L  G  L  I  K  A  L  K  H  L  G  E  P  L  A  T  M  Q  G  L  M  T  E  L  E  P  G  I  T  V

       4330      4340      4350      4360      4370      4380      4390      4400      4410
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CATGTACCCCGGAAATCCAAATTGAGAAAGACGACCGCACACGCGGTGTACAAACCGGAGTTTGAGCCTGCTGTGTTGTCAAAATTTGAT
 H  V  P  R  K  S  K  L  R  K  T  T  A  H  A  V  Y  K  P  E  F  E  P  A  V  L  S  K  F  D

       4420      4430      4440      4450      4460      4470      4480      4490      4500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CCCAGACTGAACAAGGATGTTGACTTGGATGAAGTAATTTGGTCTAAACACACTGCCAATGTCCCTTACCAACCTCCTTTGTTCTACACA
 P  R  L  N  K  D  V  D  L  D  E  V  I  W  S  K  H  T  A  N  V  P  Y  Q  P  P  L  F  Y  T
```

FIG. 5D

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        4510       4520       4530       4540       4550       4560       4570       4580       4590
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TACATGTCAGAGTACGCTCATCGAGTCTTCTCCTTCTTGGGGAAAGACAATGACATTCTGACCGTCAAAGAAGCAATTCTGGGCATCCCC
 Y  M  S  E  Y  A  H  R  V  F  S  F  L  G  K  D  N  D  I  L  T  V  K  E  A  I  L  G  I  P

        4600       4610       4620       4630       4640       4650       4660       4670       4680
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGACTAGACCCCATGGATCCCCACACAGCTCCGGGTCTGCCTTACGCCATCAACGGCCTTCGACGTACTGATCTCGTCGATTTTGTGAAC
 G  L  D  P  M  D  P  H  T  A  P  G  L  P  Y  A  I  N  G  L  R  R  T  D  L  V  D  F  V  N

        4690       4700       4710       4720       4730       4740       4750       4760       4770
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGTACAGTAGATGCGGCGCTGGCTGTACAAATCCAGAAATTCTTAGACGGTGACTACTCTGACCATGTCTTCCAAACTTTTCTGAAAGAT
 G  T  V  D  A  A  L  A  V  Q  I  Q  K  F  L  D  G  D  Y  S  D  H  V  F  Q  T  F  L  K  D

        4780       4790       4800       4810       4820       4830       4840       4850       4860
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAGATCAGACCCTCAGAGAAAGTCCGAGCGGGAAAAACCCGCATTGTTGATGTGCCCTCCCTGGCGCATTGCATTGTGGGCAGAATGTTG
 E  I  R  P  S  E  K  V  R  A  G  K  T  R  I  V  D  V  P  S  L  A  H  C  I  V  G  R  M  L

        4870       4880       4890       4900       4910       4920       4930       4940       4950
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTTGGGCGCTTTGCTGCCAAGTTTCAATCCCATCCTGGCTTTCTCCTCGGCTCTGCTATCGGGTCTGACCCTGATGTTTTCTGGACCGTC
 L  G  R  F  A  A  K  F  Q  S  H  P  G  F  L  L  G  S  A  I  G  S  D  P  D  V  F  W  T  V

        4960       4970       4980       4990       5000       5010       5020       5030       5040
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ATAGGGGCTCAACTCGAGGGGAGAAAGAACACGTATGACGTGGACTACAGTGCCTTTGACTCTTCACACGGCACTGGCTCCTTCGAGGCT
 I  G  A  Q  L  E  G  R  K  N  T  Y  D  V  D  Y  S  A  F  D  S  S  H  G  T  G  S  F  E  A

        5050       5060       5070       5080       5090       5100       5110       5120       5130
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CTCATCTCTCACTTTTTCACCGTGGACAATGGTTTTAGCCCTGCGCTGGGACCGTATCTCAGATCCCTGGCTGTCTCGGTGCACGCTTAC
 L  I  S  H  F  F  T  V  D  N  G  F  S  P  A  L  G  P  Y  L  R  S  L  A  V  S  V  H  A  Y

        5140       5150       5160       5170       5180       5190       5200       5210       5220
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GGCGAGCGTCGCATCAAGATTACCGGTGGCCTCCCCTCCGGTTGTGCCGCGACCAGCCTGCTGAACACAGTGCTCAACAATGTGATCATC
 G  E  R  R  I  K  I  T  G  G  L  P  S  G  C  A  A  T  S  L  L  N  T  V  L  N  N  V  I  I

        5230       5240       5250       5260       5270       5280       5290       5300       5310
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AGGACTGCTCTGGCATTGACTTACAAGGAATTTGAGTATGACACGGTTGATATCATCGCCTACGGTGACGACCTTCTGGTTGGCACGGAT
 R  T  A  L  A  L  T  Y  K  E  F  E  Y  D  T  V  D  I  I  A  Y  G  D  D  L  L  V  G  T  D

        5320       5330       5340       5350       5360       5370       5380       5390       5400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TACGATCTGGACTTCAATGAGGTGGCACGACGCGCTGCCAAGTTGGGGTATAAGATGACTCCTGCCAACAAGGGTTCTGTCTTCCCTCCG
 Y  D  L  D  F  N  E  V  A  R  R  A  A  K  L  G  Y  K  M  T  P  A  N  K  G  S  V  F  P  P

        5410       5420       5430       5440       5450       5460       5470       5480       5490
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ACTTCCTCTCTTTCCGATGCTGTTTTTCTAAAGCGCAAATTCGTCCAAAACAACGACGGCTTATACAAACCAGTTATGGATTTAAAGAAT
 T  S  S  L  S  D  A  V  F  L  K  R  K  F  V  Q  N  N  D  G  L  Y  K  P  V  M  D  L  K  N

        5500       5510       5520       5530       5540       5550       5560       5570       5580
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
TTGGAAGCCATGCTCTCCTACTTCAAACCAGGAACACTACTCGAGAAGCTGCAATCTGTTTCTATGTTGGCTCAACATTCTGGAAAAGAA
 L  E  A  M  L  S  Y  F  K  P  G  T  L  L  E  K  L  Q  S  V  S  M  L  A  Q  H  S  G  K  E

        5590       5600       5610       5620       5630       5640       5650       5660       5670
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
GAATATGATAGATTGATGCACCCCTTCGCTGACTACGGTGCCGTACCGAGTCACGAGTACCTGCAGGCAAGATGGAGGGCCTTGTTCGAC
 E  Y  D  R  L  M  H  P  F  A  D  Y  G  A  V  P  S  H  E  Y  L  Q  A  R  W  R  A  L  F  D

        5680       5690       5700       5710       5720       5730       5740       5750
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|..
TGACCCAGATAGCCCAAGGCGCTTCGGTGCTGCCGGCGATTCTGGGAGAACTCAGTCGGAACAGAAAAAAAAAAAAAAAAAA
 *
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FIG. 5E

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NNTCTAGCCC ACCATGGCAA CAAGAAGAGC TTACAGGAGC TGAATGAAGA ACAGTGGGTG 60
GAAATGTCTG ACGATTACCG GACCGGGAAA AACATGCCTT TTCAGTCTCT TGGCACATAC 120
TATCGGCCCC CTAACTGGAC TTGGGGTCCC AATTTCATCA ACCCCTATCA AGTAACGGTT 180
TTCCCACACC AAATTCTGAA CGCGAGAACC TCTACCTCGG TAGACATAAA CGTCCCATAC 240
ATCGGGGAGA CCCCCACGCA ATCCTCAGAG ACACAGAACT CCTGGACCCT CCTCGTTATG 300
GTGCTCGTTC CCCTAGACTA TAAGGAAGGA GCCACAACTG ACCCAGAAAT TACATTTTCT 360
GTAAGGCCTA CAAGTCCCTA CTTCAATGGG CTTCGCAACC GCTACACGGC CGGGACGGAC 420
GAAGAACAGG GGCCCATTCC TACGGCACCC AGAGAAAATT CGCTTATGTT TCTCTCAACC 480
CTCCCTGACG ACACTGTCCC TGCTTACGGG AATGTGCGTA CCCCTCCTGT CAATTACCTC 540
CCTGGTGAAA TAACCGACCT TTTGCAACTG GCCCGCATAC CCACTCTCAT GGCATTTGAG 600
CGGGTGCCTG AACCCGTGCC TGCCTCAGAC ACATATGTGC CCTACGTTGC CGTTCCCACC 660
CAGTTCGATG ACAGGCCTCT CATCTCCTTC CCGATCACCC TTTCAGATCC CGTCTATCAG 720
AACACCCTGG TTGGCGCCAT CAGTTCAAAT TTCGCCAATT ACCGTGGGTG TATCCAAATC 780
ACTCTGACAT TTTGTGGACC CATGATGGCG AGAGGGAAAT TCCTGCTCTC GTATTCTCCC 840
CCAAATGGAA CGCAACCACA GACTCTTTCC GAAGCTATGC AGTGCACATA CTCTATTTGG 900
GACATAGGCT TGAACTCTAG TTGGACCTTC GTCGTCCCCT ACATCTCGCC CAGTGACTAC 960
CGTGAAACTC GAGCCATTAC CAACTCGGTT TACTCCGCTG ATGGTTGGTT TAGCCTGCAC 1020
AAGTTGACCA AAATTACTCT ACCACCTGAC TGTCCGCAAA GTCCCTGCAT TCTCTTTTTC 1080
GCTTCTGCTG GTGAGGATTA CACTCTCCGT CTCCCCGTTG ATTGTAATCC TTCCTATGTG 1140
TTCCACTCCA CCGACAACGC CGAGACCGGG GTTATTGAGG CGGGTAACAC TGACACCGAT 1200
TTCTCTGGTG AACTGGCGGC TCCTGGCCCT AACCACACTA ATGTCAAGTT CCTGTTTGAT 1260
CGATCTCGAT TATTGAATGT AATCAAGGTA CTGGAGAAGG ACGCCGTTTT CCCCCGCCCT 1320
TTCCCTACAC AAGAAGGTGC GCAGCAGGAT GATGGTTACT TTTGTCTTCT GACCCCCCGC 1380
CCAACAGTCG CTTCCCGACC CGCCACTCGT TTCGGCCTGT ACGCCAATCC GTCCGGCAGT 1440
GGTGTTCTTG CTAACACTTC ACTGGACTTC AATTTTTATA GCTTGGCCTG TTTCACTTAC 1500
```

FIG. 6A

TTTAGATCGG ACCTTGAGGT TACGGTGGTC TCACTAGAGC CGGATCTGGA ATTTGCTGTA 1560

GGGTGGTTTC CTTCTGGCAG TGAATACCAG GCTTCCAGCT TTGTCTACGA CCAGCTGCAT 1620

GTGCCCTTCC ACTTTACTGG GCGCACTCCC CGCGCTTTCG CTAGCAAGGG TGGGAAGGTA 1680

TCTTTCGTGC TCCCTTGGAA CTCTGTCTCG TCTGTGCTCC CCGTGCGCTG GGGGGGGGCT 1740

TCCAAGCTCT CTTCTGCTAC GCGGGGTCTA CCGGCGCATG CTGATTGGGG GACTATTTAC 1800

GCCTTTGTCC CCCGTCCTAA TGAGAAGAAA AGCACCGCTG TAAAACACGT GGCCGTGTAC 1860

ATTCGGTACA AGAACGCACG TGCCTGGTGC CCCAGCATGC TTCCCTTTCG CAGCTACAAG 1920

CAGAAGATGC TGATGCAATC TGGCGATATC GAGACCAATC CTGGTCCTGC TTCTGACAAC 1980

CCAATTTTGG AGTTTCTTGA AGCAGAAAAT GATCTAGTCA CTCTGGCCTC TCTCTGGAAG 2040

ATGGTGCACT CTGTTCAACA GACCTGGAGA AAGTATGTGA AGAACGATGA TTTTTGGCCC 2100

AATTTACTCA GCGAGCTAGT GGGGGAAGGC TCTGTCGCCT TGGCCGCCAC GCTATCCAAC 2160

CAAGCTTCAG TAAAGGCTCT TTTGGGCCTG CACTTTCTCT CTCGGGGGCT CAATTACACT 2220

GACTTTTACT CTTTACTGAT AGAGAAATGC TCTAGTTTCT TTACCGTAGA ACCACCTCCT 2280

CCACCAGCTG AAAACCTGAT GACCAAGCCC TCAGTGAAGT CGAAATTCCG AAAACTGTTT 2340

AAGATGCAAG GACCCATGGA CAAAGTCAAA GACTGGAACC AAATAGCTGC CGGCTTGAAG 2400

AATTTTCAAT TTGTTCGTGA CCTAGTCAAA GAGGTGGTCG ATTGGCTGCA GGCCTGGATC 2460

AACAAAGAGA AAGCCAGCCC TGTCCTCCAG TACCAGTTGG AGATGAAGAA GCTCGGGCCT 2520

GTGGCCTTGG CTCATGACGC TTTCATGGCT GGTTCCGGGC CCCCTCTTAG CGACGACCAG 2580

ATTGAATACC TCCAGAACCT CAAATCTCTT GCCCTAACAC TGGGGAAGAC TAATTTGGCC 2640

CAAAGTCTCA CCACTATGAT CAATGCCAAA CAAAGTTCAG CCCAACGAGT TGAACCCGTT 2700

GTGGTGGTCC TTAGAGGCAA GCCGGGATGC GGCAAGGGCT TGGCCTCTAC GTTGATTGCC 2760

CAGGCTGTGT CCAAGCGCCT CTATGGCTCC CAAAGTGTAT ATTCTCTTCC CCCAGATCCA 2820

GATTTCTTCG ATGGATACAA AGGACAGTTC GTGACCTTGA TGGATGATTT GGGACAAAAC 2880

CCGGATGGAC AAGATTTCCC CACCTTTTGT CAGATGGTGT CGACCGCCCA ATTTCTCCCC 2940

AACATGGCGG ACCTTGCAGA GAAAGGGCGT CCCTTTACCT CCAATCTCAT CATTGCAACT 3000

FIG. 6B

ACAAATCTCC CCCACTTCAG TCCTGTCACC ATTGCTGATC CTTCTGCAGT CTCTCGCCGT 3060

ATCAACTACG ATCTGACTCT AGAAGTATCT GAGGCCTACA AGAAACACAC ACGGCTGAAT 3120

TTTGACTTGG CTTTCAGGCG CACAGACGCC CCCCCCATTT ATCCTTTTGC TGCCCATGTG 3180

CCCTTTGTGG ACGTAGCTGT GCGCTTCAAA AATGGTCACC AGAATTTTAA TCTCCTAGAG 3240

TTGGTCGATT CCATTTGTAC AGACATTCGA GCCAAGCAAC AAGGTGCCCG AAACATGCAG 3300

ACTCTGGTTC TACAGAGCCC CAACGAGAAT GATGACACCC CCGTCGACGA GGCGTTGGGT 3360

AGAGTTCTCT CCCCCGCTGC GGTCGATGAG GCGCTTGTCG ACCTCACTCC AGAGGCCGAC 3420

CCGGTTGGCC GTTTGGCTAT TCTTGCCAAG CTAGGTCTTG CCCTAGCTGC GGTCACCCCT 3480

GGTCTGATAA TCTTGGCAGT GGGACTCTAC AGGTACTTCT CTGGCTCTGA TGCAGACCAA 3540

GAAGAAACAG AAAGTGAGGG ATCTGTCAAG GCACCCAGGA GCGAAAATGC TTATGACGGC 3600

CCGAAGAAAA ACTCTAAGCC CCCTGGAGCA CTCTCTCTCA TGGAAATGCA ACAGCCCAAC 3660

GTGGACATGG GCTTTGAGGC TGCGGTCGCT AAGAAAGTGG TCGTCCCCAT TACCTTCATG 3720

GTTCCCAACA GACCTTCTGG GCTTACACAG TCCGCTCTTC TGGTGACCGG CCGGACCTTC 3780

CTAATCAATG AACATACATG GTCCAATCCC TCCTGGACCA GCTTCACAAT CCGCGGTGAG 3840

GTACACACTC GTGATGAGCC CTTCCAAACG GTTCATTTCA CTCACCACGG TATTCCCACA 3900

GATCTGATGA TGGTACGTCT CGGACCGGGC AATTCTTTCC CTAACAATCT AGACAAGTTT 3960

GGACTTGACC AGATGCCGGC ACGCAACTCC CGTGTGGTTG GCGTTTCGTC CAGTTACGGA 4020

AACTTCTTCT TCTCTGGAAA TTTCCTCGGA TTTGTTGATT CCGTCACCTC TGAACAAGGA 4080

ACTTACGCAA GACTCTTTAG GTACAGGGTG ACGACCTACA AAGGATGGTG CGGCTCGGCC 4140

CTGGTCTGTG AGGCCGGTGG CGTCCGACGC ATCATTGGCC TGCATTCTGC TGGCGCCGCC 4200

GGTATCGGCG CCGGGACCTA TATCTCAAAA TTAGGACTAA TCAAAGCCCT GAAACACCTC 4260

GGTGAACCTT TGGCCACAAT GCAAGGACTG ATGACTGAAT TAGAGCCTGG AATCACCGTA 4320

CATGTACCCC GGAAATCCAA ATTGAGAAAG ACGACCGCAC ACGCGGTGTA CAAACCGGAG 4380

TTTGAGCCTG CTGTGTTGTC AAAATTTGAT CCCAGACTGA ACAAGGATGT TGACTTGGAT 4440

GAAGTAATTT GGTCTAAACA CACTGCCAAT GTCCCTTACC AACCTCCTTT GTTCTACACA 4500

FIG. 6C

TACATGTCAG AGTACGCTCA TCGAGTCTTC TCCTTCTTGG GGAAAGACAA TGACATTCTG 4560

ACCGTCAAAG AAGCAATTCT GGGCATCCCC GGACTAGACC CCATGGATCC CCACACAGCT 4620

CCGGGTCTGC CTTACGCCAT CAACGGCCTT CGACGTACTG ATCTCGTCGA TTTTGTGAAC 4680

GGTACAGTAG ATGCGGCGCT GGCTGTACAA ATCCAGAAAT TCTTAGACGG TGACTACTCT 4740

GACCATGTCT TCCAAACTTT TCTGAAAGAT GAGATCAGAC CCTCAGAGAA AGTCCGAGCG 4800

GGAAAAACCC GCATTGTTGA TGTGCCCTCC CTGGCGCATT GCATTGTGGG CAGAATGTTG 4860

CTTGGGCGCT TTGCTGCCAA GTTTCAATCC CATCCTGGCT TTCTCCTCGG CTCTGCTATC 4920

GGGTCTGACC CTGATGTTTT CTGGACCGTC ATAGGGGCTC AACTCGAGGG GAGAAAGAAC 4980

ACGTATGACG TGGACTACAG TGCCTTTGAC TCTTCACACG GCACTGGCTC CTTCGAGGCT 5040

CTCATCTCTC ACTTTTTCAC CGTGGACAAT GGTTTTAGCC CTGCGCTGGG ACCGTATCTC 5100

AGATCCCTGG CTGTCTCGGT GCACGCTTAC GGCGAGCGTC GCATCAAGAT TACCGGTGGC 5160

CTCCCCTCCG GTTGTGCCGC GACCAGCCTG CTGAACACAG TGCTCAACAA TGTGATCATC 5220

AGGACTGCTC TGGCATTGAC TTACAAGGAA TTTGAGTATG ACACGGTTGA TATCATCGCC 5280

TACGGTGACG ACCTTCTGGT TGGCACGGAT TACGATCTGG ACTTCAATGA GGTGGCACGA 5340

CGCGCTGCCA AGTTGGGGTA TAAGATGACT CCTGCCAACA AGGGTTCTGT CTTCCCTCCG 5400

ACTTCCTCTC TTTCCGATGC TGTTTTTCTA AAGCGCAAAT TCGTCCAAAA CAACGACGGC 5460

TTATACAAAC CAGTTATGGA TTTAAAGAAT TTGGAAGCCA TGCTCTCCTA CTTCAAACCA 5520

GGAACACTAC TCGAGAAGCT GCAATCTGTT TCTATGTTGG CTCAACATTC TGGAAAAGAA 5580

GAATATGATA GATTGATGCA CCCCTTCGCT GACTACGGTG CCGTACCGAG TCACGAGTAC 5640

CTGCAGGCAA GATGGAGGGC CTTGTTCGAC TGACCCAGAT AGCCCAAGGC GCTTCGGTGC 5700

TGCCGGCGAT TCTGGGAGAA CTCAGTCGGA ACAGAAAAAA AAAAAAAAAA AA 5752

(SEQ ID NO:1)

FIG. 6D

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XLAHHGNKKS LQELNEEQWV EMSDDYRTGK NMPFQSLGTY YRPPNWTWGP NFINPYQVTV 60
FPHQILNART STSVDINVPY IGETPTQSSE TQNSWTLLVM VLVPLDYKEG ATTDPEITFS 120
VRPTSPYFNG LRNRYTAGTD EEQGPIPTAP RENSLMFLST LPDDTVPAYG NVRTPPVNYL 180
PGEITDLLQL ARIPTLMAFE RVPEPVPASD TYVPYVAVPT QFDDRPLISF PITLSDPVYQ 240
NTLVGAISSN FANYRGCIQI TLTFCGPMMA RGKFLLSYSP PNGTQPQTLS EAMQCTYSIW 300
DIGLNSSWTF VVPYISPSDY RETRAITNSV YSADGWFSLH KLTKITLPPD CPQSPCILFF 360
ASAGEDYTLR LPVDCNPSYV FHSTDNAETG VIEAGNTDTD FSGELAAPGP NHTNVKFLFD 420
RSRLLNVIKV LEKDAVFPRP FPTQEGAQQD DGYFCLLTPR PTVASRPATR FGLYANPSGS 480
GVLANTSLDF NFYSLACFTY FRSDLEVTVV SLEPDLEFAV GWFPSGSEYQ ASSFVYDQLH 540
VPFHFTGRTP RAFASKGGKV SFVLPWNSVS SVLPVRWGGA SKLSSATRGL PAHADWGTIY 600
AFVPRPNEKK STAVKHVAVY IRYKNARAWC PSMLPFRSYK QKMLMQSGDI ETNPGPASDN 660
PILEFLEAEN DLVTLASLWK MVHSVQQTWR KYVKNDDFWP NLLSELVGEG SVALAATLSN 720
QASVKALLGL HFLSRGLNYT DFYSLLIEKC SSFFTVEPPP PPAENLMTKP SVKSKFRKLF 780
KMQGPMDKVK DWNQIAAGLK NFQFVRDLVK EVVDWLQAWI NKEKASPVLQ YQLEMKKLGP 840
VALAHDAFMA GSGPPLSDDQ IEYLQNLKSL ALTLGKTNLA QSLTTMINAK QSSAQRVEPV 900
VVVLRGKPGC GKGLASTLIA QAVSKRLYGS QSVYSLPPDP DFFDGYKGQF VTLMDDLGQN 960
PDGQDFSTFC QMVSTAQFLP NMADLAEKGR PFTSNLIIAT TNLPHFSPVT IADPSAVSRR 1020
INYDLTLEVS EAYKKHTRLN FDLAFRRTDA PPIYPFAAHV PFVDVAVRFK NGHQNFNLLE 1080
LVDSICTDIR AKQQGARNMQ TLVLQSPNEN DDTPVDEALG RVLSPAAVDE ALVDLTPEAD 1140
PVGRLAILAK LGLALAAVTP GLIILAVGLY RYFSGSDADQ EETESEGSVK APRSENAYDG 1200
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FIG. 7A

PKKNSKPPGA LSLMEMQQPN VDMGFEAAVA KKVVVPITFM VPNRPSGLTQ SALLVTGRTF 1260

LINEHTWSNP SWTSFTIRGE VHTRDEPFQT VHFTHHGIPT DLMMVRLGPG NSFPNNLDKF 1320

GLDQMPARNS RVVGVSSSYG NFFFSGNFLG FVDSVTSEQG TYARLFRYRV TTYKGWCGSA 1380

LVCEAGGVRR IIGLHSAGAA GIGAGTYISK LGLIKALKHL GEPLATMQGL MTELEPGITV 1440

HVPRKSKLRK TTAHAVYKPE FEPAVLSKFD PRLNKDVDLD EVIWSKHTAN VPYQPPLFYT 1500

YMSEYAHRVF SFLGKDNDIL TVKEAILGIP GLDPMDPHTA PGLPYAINGL RRTDLVDFVN 1560

GTVDAALAVQ IQKFLDGDYS DHVFQTFLKD EIRPSEKVRA GKTRIVDVPS LAHCIVGRML 1620

LGRFAAKFQS HPGFLLGSAI GSDPDVFWTV IGAQLEGRKN TYDVDYSAFD SSHGTGSFEA 1680

LISHFFTVDN GFSPALGPYL RSLAVSVHAY GERRIKITGG LPSGCAATSL LNTVLNNVII 1740

RTALALTYKE FEYDTVDIIA YGDDLLVGTD YDLDFNEVAR RAAKLGYKMT PANKGSVFPP 1800

TSSLSDAVFL KRKFVQNNDG LYKPVMDLKN LEAMLSYFKP GTLLEKLQSV SMLAQHSGKE 1860

EYDRLMHPFA DYGAVPSHEY LQARWRALFD * (SEQ ID NO:2) 1890

FIG. 7B

CTAGCCCACC ATGGCAACAA GAAGAGCTTA CAGGAGCTGA ATGAAGAACA GTGGGTGGAA 60

ATGTCTGACG ATTACCGGAC CGGGAAAAAC ATGCCTTTTC AGTCTCTTGG CACATACTAT 120

CGGCCCCCTA ACTGGACTTG GGGTCCCAAT TTCATCAACC CCTATCAAGT AACGGTTTTC 180

CCACACCAAA TTCTGAACGC GAGAACCTCT ACCTCGGTAG ACATAAACGT CCCATACATC 240

GGGGAGACCC CCACGCAATC CTCAGAGACA CAGAACTCCT GGACCCTCCT CGTTATGGTG 300

CTCGTTCCCC TAGACTATAA GGAAGGAGCC ACAACTGACC CAGAAATTAC ATTTTCTGTA 360

AGGCCTACAA GTCCCTACTT CAATGGGCTT CGCAACCGCT ACACGGCCGG GACGGACGAA 420

GAACAG (SEQ ID NO:3) 426

FIG. 8

LAHHGNKKSL QELNEEQWVE MSDDYRTGKN MPFQSLGTYY RPPNWTWGPN FINPYQVTVF 60

PHQILNARTS TSVDINVPYI GETPTQSSET QNSWTLLVMV LVPLDYKEGA TTDPEITFSV 120

RPTSPYFNGL RNRYTAGTDE EQ (SEQ ID NO:4) 142

FIG. 9

GGGCCCATTC CTACGGCACC CAGAGAAAAT TCGCTTATGT TTCTCTCAAC CCTCCCTGAC 60

GACACTGTCC CTGCTTACGG GAATGTGCGT ACCCCTCCTG TCAATTACCT CCCTGGTGAA 120

ATAACCGACC TTTTGCAACT GGCCCGCATA CCCACTCTCA TGGCATTTGA GCGGGTGCCT 180

GAACCCGTGC CTGCCTCAGA CACATATGTG CCCTACGTTG CCGTTCCCAC CCAGTTCGAT 240

GACAGGCCTC TCATCTCCTT CCCGATCACC CTTTCAGATC CCGTCTATCA GAACACCCTG 300

GTTGGCGCCA TCAGTTCAAA TTTCGCCAAT TACCGTGGGT GTATCCAAAT CACTCTGACA 360

TTTTGTGGAC CCATGATGGC GAGAGGGAAA TTCCTGCTCT CGTATTCTCC CCCAAATGGA 420

ACGCAACCAC AGACTCTTTC CGAAGCTATG CAGTGCACAT ACTCTATTTG GGACATAGGC 480

TTGAACTCTA GTTGGACCTT CGTCGTCCCC TACATCTCGC CCAGTGACTA CCGTGAAACT 540

CGAGCCATTA CCAACTCGGT TTACTCCGCT GATGGTTGGT TTAGCCTGCA CAAGTTGACC 600

AAAATTACTC TACCACCTGA CTGTCCGCAA AGTCCCTGCA TTCTCTTTTT CGCTTCTGCT 660

GGTGAGGATT ACACTCTCCG TCTCCCCGTT GATTGTAATC CTTCCTATGT GTTCCAC 717

(SEQ ID NO:5)

FIG. 10

GPIPTAPREN SLMFLSTLPD DTVPAYGNVR TPPVNYLPGE ITDLLQLARI PTLMAFERVP 60

EPVPASDTYV PYVAVPTQFD DRPLISFPIT LSDPVYQNTL VGAISSNFAN YRGCIQITLT 120

FCGPMMARGK FLLSYSPPNG TQPQTLSEAM QCTYSIWDIG LNSSWTFVVP YISPSDYRET 180

RAITNSVYSA DGWFSLHKLT KITLPPDCPQ SPCILFFASA GEDYTLRLPV DCNPSYVFH 239

(SEQ ID NO:6)

FIG. 11

TCCACCGACA ACGCCGAGAC CGGGGTTATT GAGGCGGGTA ACACTGACAC CGATTTCTCT 60

GGTGAACTGG CGGCTCCTGG CCCTAACCAC ACTAATGTCA AGTTCCTGTT TGATCGATCT 120

CGATTATTGA ATGTAATCAA GGTACTGGAG AAGGACGCCG TTTTCCCCCG CCCTTTCCCT 180

ACACAAGAAG GTGCGCAGCA GGATGATGGT TACTTTTGTC TTCTGACCCC CCGCCCAACA 240

GTCGCTTCCC GACCCGCCAC TCGTTTCGGC CTGTACGCCA ATCCGTCCGG CAGTGGTGTT 300

CTTGCTAACA CTTCACTGGA CTTCAATTTT TATAGCTTGG CCTGTTTCAC TTACTTTAGA 360

TCGGACCTTG AGGTTACGGT GGTCTCACTA GAGCCGGATC TGGAATTTGC TGTAGGGTGG 420

TTTCCTTCTG GCAGTGAATA CCAGGCTTCC AGCTTTGTCT ACGACCAGCT GCATGTGCCC 480

TTCCACTTTA CTGGGCGCAC TCCCCGCGCT TTCGCTAGCA AGGGTGGGAA GGTATCTTTC 540

GTGCTCCCTT GGAACTCTGT CTCGTCTGTG CTCCCCGTGC GCTGGGGGGG GGCTTCCAAG 600

CTCTCTTCTG CTACGCGGGG TCTACCGGCG CATGCTGATT GGGGGACTAT TTACGCCTTT 660

GTCCCCCGTC CTAATGAGAA GAAAAGCACC GCTGTAAAAC ACGTGGCCGT GTACATTCGG 720

TACAAGAACG CACGTGCCTG GTGCCCCAGC ATGCTTCCCT TTCGCAGCTA CAAGCAG 777

(SEQ ID NO:7)

FIG. 12

STDNAETGVI EAGNTDTDFS GELAAPGPNH TNVKFLFDRS RLLNVIKVLE KDAVFPRPFP 60

TQEGAQQDDG YFCLLTPRPT VASRPATRFG LYANPSGSGV LANTSLDFNF YSLACFTYFR 120

SDLEVTVVSL EPDLEFAVGW FPSGSEYQAS SFVYDQLHVP FHFTGRTPRA FASKGGKVSF 180

VLPWNSVSSV LPVRWGGASK LSSATRGLPA HADWGTIYAF VPRPNEKKST AVKHVAVYIR 240

YKNARAWCPS MLPFRSYKQ (SEQ ID NO:8) 259

FIG. 13

AAGATGCTGA TGCAATCTGG CGATATCGAG ACCAATCCTG GT (SEQ ID NO:9) 42

FIG. 14

KMLMQSGDIE TNPG (SEQ ID NO:10) 14

FIG. 15

CCTGCTTCTG ACAACCCAAT TTTGGAGTTT CTTGAAGCAG AAAATGATCT AGTCACTCTG 60

GCCTCTCTCT GGAAGATGGT GCACTCTGTT CAACAGACCT GGAGAAAGTA TGTGAAGAAC 120

GATGATTTTT GGCCCAATTT ACTCAGCGAG CTAGTGGGGG AAGGCTCTGT CGCCTTGGCC 180

GCCACGCTAT CCAACCAAGC TTCAGTAAAG GCTCTTTTGG GCCTGCACTT TCTCTCTCGG 240

GGGCTCAATT ACACTGACTT TTACTCTTTA CTGATAGAGA AATGCTCTAG TTTCTTTACC 300

GTAGAACCAC CTCCTCCACC AGCTGAAAAC CTGATGACCA AGCCCTCAGT GAAGTCGAAA 360

TTCCGAAAAC TGTTTAAGAT GCAA (SEQ ID NO:11) 384

FIG. 16

PASDNPILEF LEAENDLVTL ASLWKMVHSV QQTWRKYVKN DDFWPNLLSE LVGEGSVALA 60

ATLSNQASVK ALLGLHFLSR GLNYTDFYSL LIEKCSSFFT VEPPPPPAEN LMTKPSVKSK 120

FRKLFKMQ (SEQ ID NO:12) 128

FIG. 17

GGACCCATGG ACAAAGTCAA AGACTGGAAC CAAATAGCTG CCGGCTTGAA GAATTTTCAA 60

TTTGTTCGTG ACCTAGTCAA AGAGGTGGTC GATTGGCTGC AGGCCTGGAT CAACAAAGAG 120

AAAGCCAGCC CTGTCCTCCA GTACCAGTTG GAGATGAAGA AGCTCGGGCC TGTGGCCTTG 180

GCTCATGACG CTTTCATGGC TGGTTCCGGG CCCCCTCTTA GCGACGACCA GATTGAATAC 240

CTCCAGAACC TCAAATCTCT TGCCCTAACA CTGGGGAAGA CTAATTTGGC CCAAAGTCTC 300

ACCACTATGA TCAATGCCAA ACAAAGTTCA GCCCAACGAG TTGAACCCGT TGTGGTGGTC 360

CTTAGAGGCA AGCCGGGATG CGGCAAGGGC TTGGCCTCTA CGTTGATTGC CCAGGCTGTG 420

TCCAAGCGCC TCTATGGCTC CCAAAGTGTA TATTCTCTTC CCCCAGATCC AGATTTCTTC 480

GATGGATACA AAGGACAGTT CGTGACCTTG ATGGATGATT TGGGACAAAA CCCGGATGGA 540

CAAGATTTCC CCACCTTTTG TCAGATGGTG TCGACCGCCC AATTTCTCCC CAACATGGCG 600

GACCTTGCAG AGAAAGGGCG TCCCTTTACC TCCAATCTCA TCATTGCAAC TACAAATCTC 660

CCCCACTTCA GTCCTGTCAC CATTGCTGAT CCTTCTGCAG TCTCTCGCCG TATCAACTAC 720

GATCTGACTC TAGAAGTATC TGAGGCCTAC AAGAAACACA CACGGCTGAA TTTTGACTTG 780

GCTTTCAGGC GCACAGACGC CCCCCCCATT TATCCTTTTG CTGCCCATGT GCCCTTTGTG 840

GACGTAGCTG TGCGCTTCAA AAATGGTCAC CAGAATTTTA ATCTCCTAGA GTTGGTCGAT 900

TCCATTTGTA CAGACATTCG AGCCAAGCAA CAAGGTGCCC GAAACATGCA GACTCTGGTT 960

CTACAG (SEQ ID NO:13) 966

FIG. 18

GPMDKVKDWN QIAAGLKNFQ FVRDLVKEVV DWLQAWINKE KASPVLQYQL EMKKLGPVAL 60

AHDAFMAGSG PPLSDDQIEY LQNLKSLALT LGKTNLAQSL TTMINAKQSS AQRVEPVVVV 120

LRGKPGCGKG LASTLIAQAV SKRLYGSQSV YSLPPDPDFF DGYKGQFVTL MDDLGQNPDG 180

QDFSTFCQMV STAQFLPNMA DLAEKGRPFT SNLIIATTNL PHFSPVTIAD PSAVSRRINY 240

DLTLEVSEAY KKHTRLNFDL AFRRTDAPPI YPFAAHVPFV DVAVRFKNGH QNFNLLELVD 300

SICTDIRAKQ QGARNMQTLV LQ (SEQ ID NO:14) 322

FIG. 19

AGCCCCAACG AGAATGATGA CACCCCCGTC GACGAGGCGT TGGGTAGAGT TCTCTCCCCC 60

GCTGCGGTCG ATGAGGCGCT TGTCGACCTC ACTCCAGAGG CCGACCCGGT TGGCCGTTTG 120

GCTATTCTTG CCAAGCTAGG TCTTGCCCTA GCTGCGGTCA CCCCTGGTCT GATAATCTTG 180

GCAGTGGGAC TCTACAGGTA CTTCTCTGGC TCTGATGCAG ACCAAGAAGA AACAGAAAGT 240

GAGGGATCTG TCAAGGCACC CAGGAGCGAA (SEQ ID NO:15) 270

FIG. 20

SPNENDDTPV DEALGRVLSP AAVDEALVDL TPEADPVGRL AILAKLGLAL AAVTPGLIIL 60

AVGLYRYFSG SDADQEETES EGSVKAPRSE (SEQ ID NO:16) 90

FIG. 21

AATGCTTATG ACGGCCCGAA GAAAAACTCT AAGCCCCCTG GAGCACTCTC TCTCATGGAA 60

ATGCAA (SEQ ID NO:17) 66

FIG. 22

NAYDGPKKNS KPPGALSLME MQ (SEQ ID NO:18) 22

FIG. 23

CAGCCCAACG TGGACATGGG CTTTGAGGCT GCGGTCGCTA AGAAAGTGGT CGTCCCCATT 60

ACCTTCATGG TTCCCAACAG ACCTTCTGGG CTTACACAGT CCGCTCTTCT GGTGACCGGC 120

CGGACCTTCC TAATCAATGA ACATACATGG TCCAATCCCT CCTGGACCAG CTTCACAATC 180

CGCGGTGAGG TACACACTCG TGATGAGCCC TTCCAAACGG TTCATTTCAC TCACCACGGT 240

ATTCCCACAG ATCTGATGAT GGTACGTCTC GGACCGGGCA ATTCTTTCCC TAACAATCTA 300

GACAAGTTTG GACTTGACCA GATGCCGGCA CGCAACTCCC GTGTGGTTGG CGTTTCGTCC 360

AGTTACGGAA ACTTCTTCTT CTCTGGAAAT TTCCTCGGAT TTGTTGATTC CGTCACCTCT 420

GAACAAGGAA CTTACGCAAG ACTCTTTAGG TACAGGGTGA CGACCTACAA AGGATGGTGC 480

GGCTCGGCCC TGGTCTGTGA GGCCGGTGGC GTCCGACGCA TCATTGGCCT GCATTCTGCT 540

GGCGCCGCCG GTATCGGCGC CGGGACCTAT ATCTCAAAAT TAGGACTAAT CAAAGCCCTG 600

AAACACCTCG GTGAACCTTT GGCCACAATG CAA (SEQ ID NO:19) 633

FIG. 24

QPNVDMGFEA AVAKKVVVPI TFMVPNRPSG LTQSALLVTG RTFLINEHTW SNPSWTSFTI 60

RGEVHTRDEP FQTVHFTHHG IPTDLMMVRL GPGNSFPNNL DKFGLDQMPA RNSRVVGVSS 120

SYGNFFFSGN FLGFVDSVTS EQGTYARLFR YRVTTYKGWC GSALVCEAGG VRRIIGLHSA 180

GAAGIGAGTY ISKLGLIKAL KHLGEPLATM Q (SEQ ID NO:20) 211

FIG. 25

GGACTGATGA CTGAATTAGA GCCTGGAATC ACCGTACATG TACCCCGGAA ATCCAAATTG 60

AGAAAGACGA CCGCACACGC GGTGTACAAA CCGGAGTTTG AGCCTGCTGT GTTGTCAAAA 120

TTTGATCCCA GACTGAACAA GGATGTTGAC TTGGATGAAG TAATTTGGTC TAAACACACT 180

GCCAATGTCC CTTACCAACC TCCTTTGTTC TACACATACA TGTCAGAGTA CGCTCATCGA 240

GTCTTCTCCT TCTTGGGGAA AGACAATGAC ATTCTGACCG TCAAAGAAGC AATTCTGGGC 300

ATCCCCGGAC TAGACCCCAT GGATCCCCAC ACAGCTCCGG GTCTGCCTTA CGCCATCAAC 360

GGCCTTCGAC GTACTGATCT CGTCGATTTT GTGAACGGTA CAGTAGATGC GGCGCTGGCT 420

GTACAAATCC AGAAATTCTT AGACGGTGAC TACTCTGACC ATGTCTTCCA AACTTTTCTG 480

AAAGATGAGA TCAGACCCTC AGAGAAAGTC CGAGCGGGAA AAACCCGCAT TGTTGATGTG 540

CCCTCCCTGG CGCATTGCAT TGTGGGCAGA ATGTTGCTTG GGCGCTTTGC TGCCAAGTTT 600

CAATCCCATC CTGGCTTTCT CCTCGGCTCT GCTATCGGGT CTGACCCTGA TGTTTTCTGG 660

ACCGTCATAG GGGCTCAACT CGAGGGGAGA AAGAACACGT ATGACGTGGA CTACAGTGCC 720

TTTGACTCTT CACACGGCAC TGGCTCCTTC GAGGCTCTCA TCTCTCACTT TTTCACCGTG 780

GACAATGGTT TTAGCCCTGC GCTGGGACCG TATCTCAGAT CCCTGGCTGT CTCGGTGCAC 840

GCTTACGGCG AGCGTCGCAT CAAGATTACC GGTGGCCTCC CCTCCGGTTG TGCCGCGACC 900

AGCCTGCTGA ACACAGTGCT CAACAATGTG ATCATCAGGA CTGCTCTGGC ATTGACTTAC 960

AAGGAATTTG AGTATGACAC GGTTGATATC ATCGCCTACG GTGACGACCT TCTGGTTGGC 1020

ACGGATTACG ATCTGGACTT CAATGAGGTG GCACGACGCG CTGCCAAGTT GGGGTATAAG 1080

ATGACTCCTG CCAACAAGGG TTCTGTCTTC CCTCCGACTT CCTCTCTTTC CGATGCTGTT 1140

TTTCTAAAGC GCAAATTCGT CCAAAACAAC GACGGCTTAT ACAAACCAGT TATGGATTTA 1200

AAGAATTTGG AAGCCATGCT CTCCTACTTC AAACCAGGAA CACTACTCGA GAAGCTGCAA 1260

TCTGTTTCTA TGTTGGCTCA ACATTCTGGA AAAGAAGAAT ATGATAGATT GATGCACCCC 1320

TTCGCTGACT ACGGTGCCGT ACCGAGTCAC GAGTACCTGC AGGCAAGATG GAGGGCCTTG 1380

TTCGACTGA (SEQ ID NO:21) 1389

FIG. 26

GLMTELEPGI TVHVPRKSKL RKTTAHAVYK PEFEPAVLSK FDPRLNKDVD LDEVIWSKHT 60

ANVPYQPPLF YTYMSEYAHR VFSFLGKDND ILTVKEAILG IPGLDPMDPH TAPGLPYAIN 120

GLRRTDLVDF VNGTVDAALA VQIQKFLDGD YSDHVFQTFL KDEIRPSEKV RAGKTRIVDV 180

PSLAHCIVGR MLLGRFAAKF QSHPGFLLGS AIGSDPDVFW TVIGAQLEGR KNTYDVDYSA 240

FDSSHGTGSF EALISHFFTV DNGFSPALGP YLRSLAVSVH AYGERRIKIT GGLPSGCAAT 300

SLLNTVLNNV IIRTALALTY KEFEYDTVDI IAYGDDLLVG TDYDLDFNEV ARRAAKLGYK 360

MTPANKGSVF PPTSSLSDAV FLKRKFVQNN DGLYKPVMDL KNLEAMLSYF KPGTLLEKLQ 420

SVSMLAQHSG KEEYDRLMHP FADYGAVPSH EYLQARWRAL FD * (SEQ ID NO:22) 462

FIG. 27

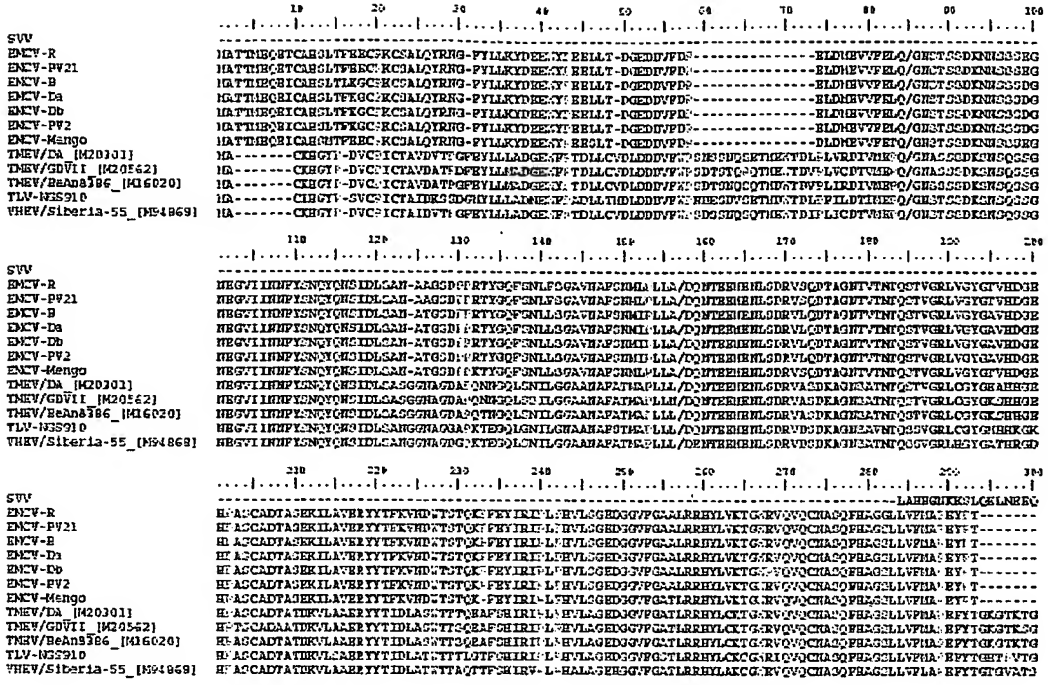
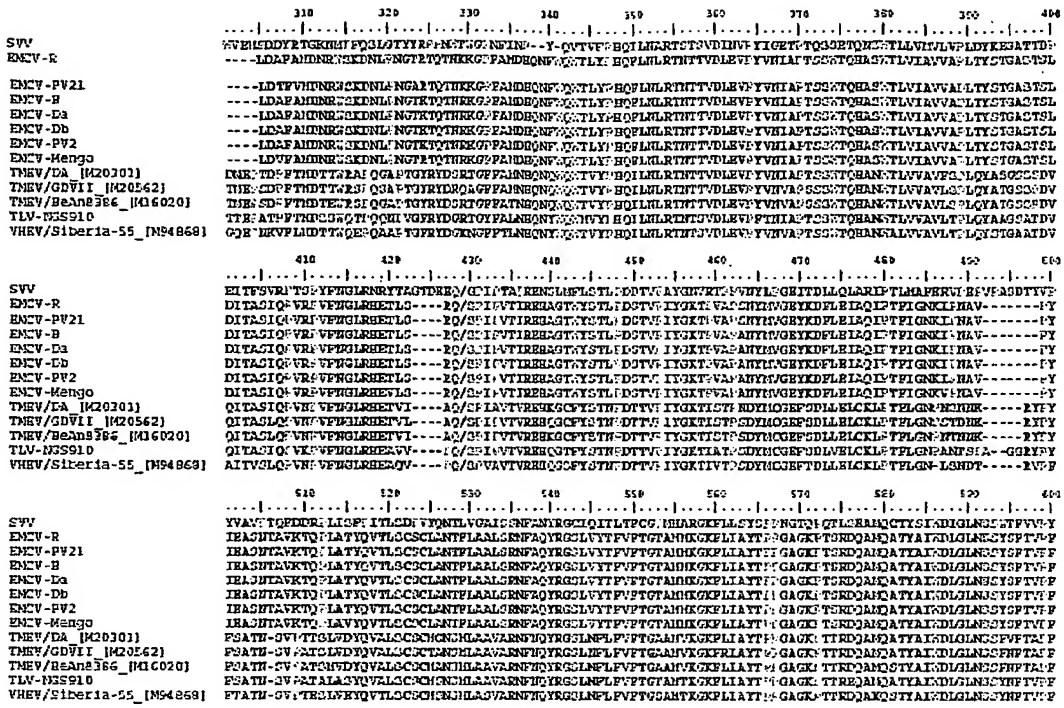


Fig. 28A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EMCV-PV21
EMCV-B
EMCV-Da
EMCV-Db
EMCV-PV2
EMCV-Mengo
TMEV/DA_[M20301]
TMEV/GDVII_[M20562]
TMEV/BeAn8386_[M16020]
TLV-NSS910
VHEV/Siberia-55_[M94869]

Fig. 28B

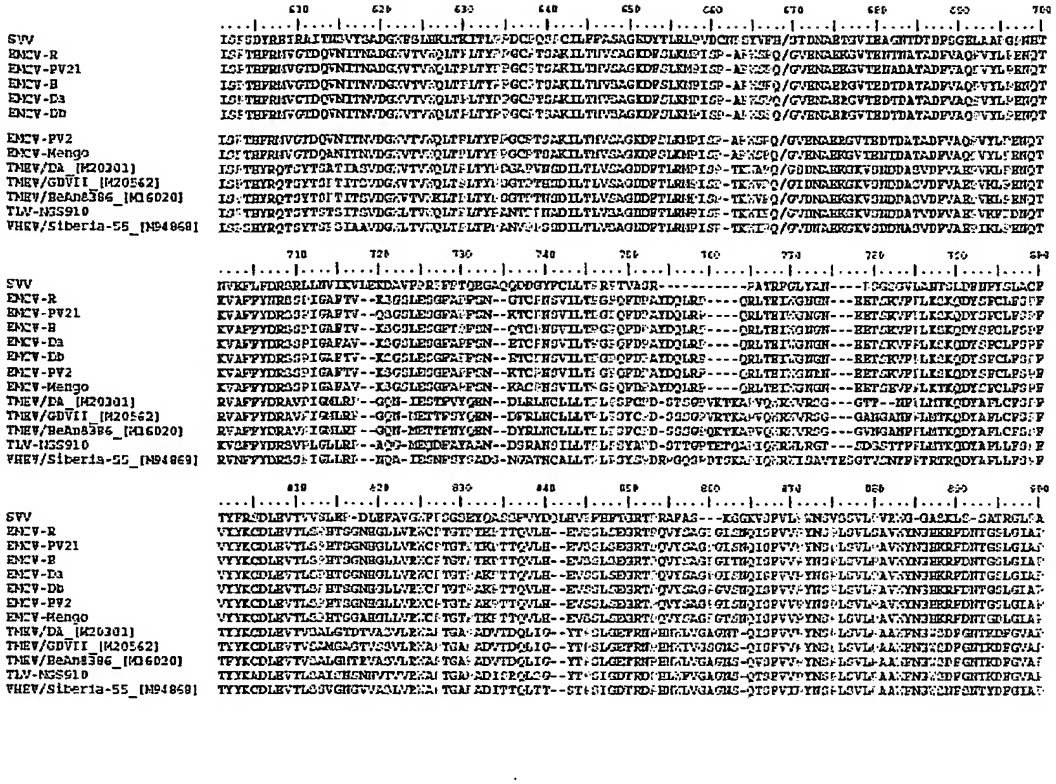


Fig. 28C

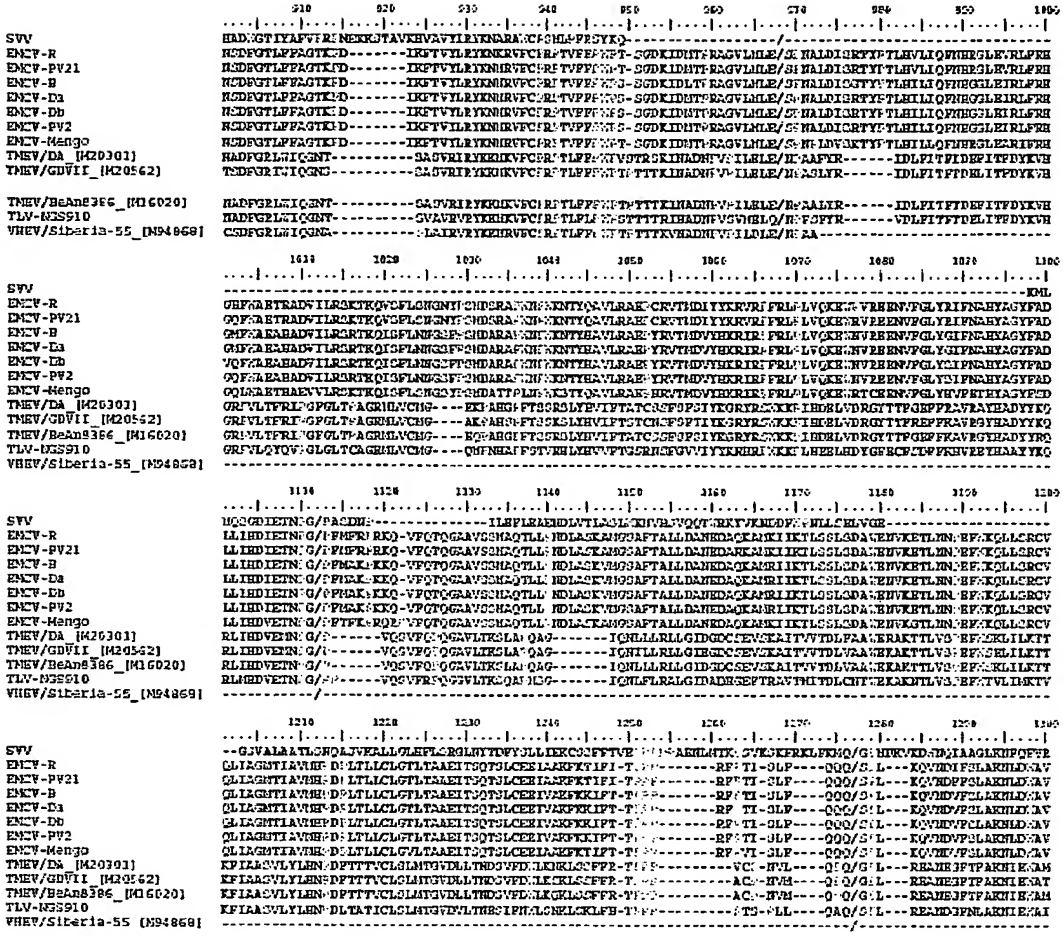


Fig. 28D

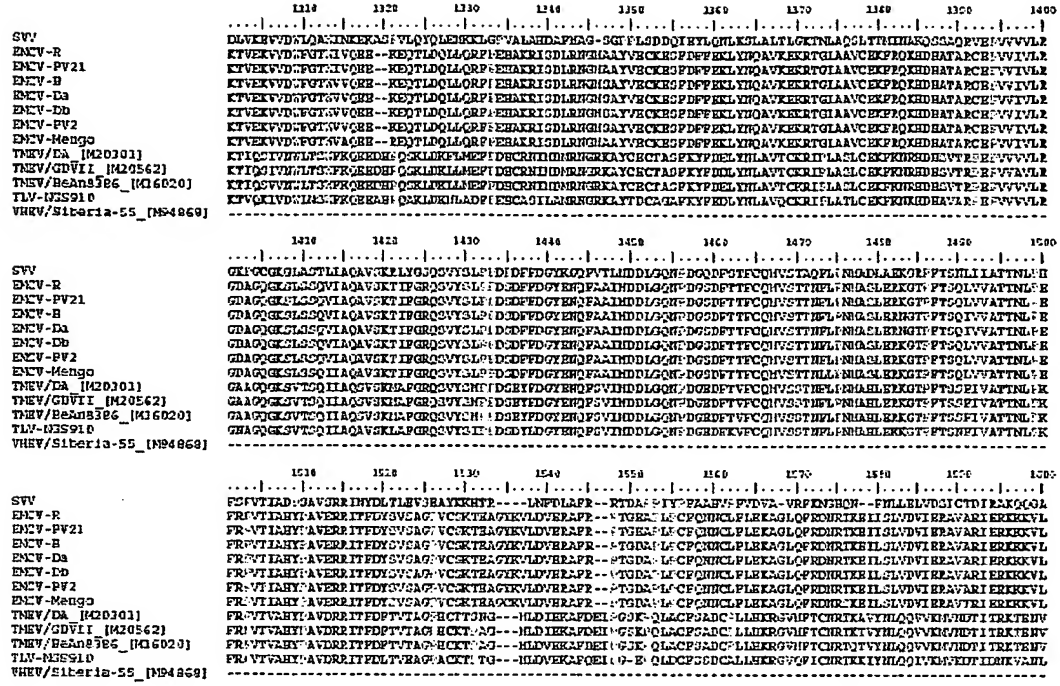


Fig. 28E

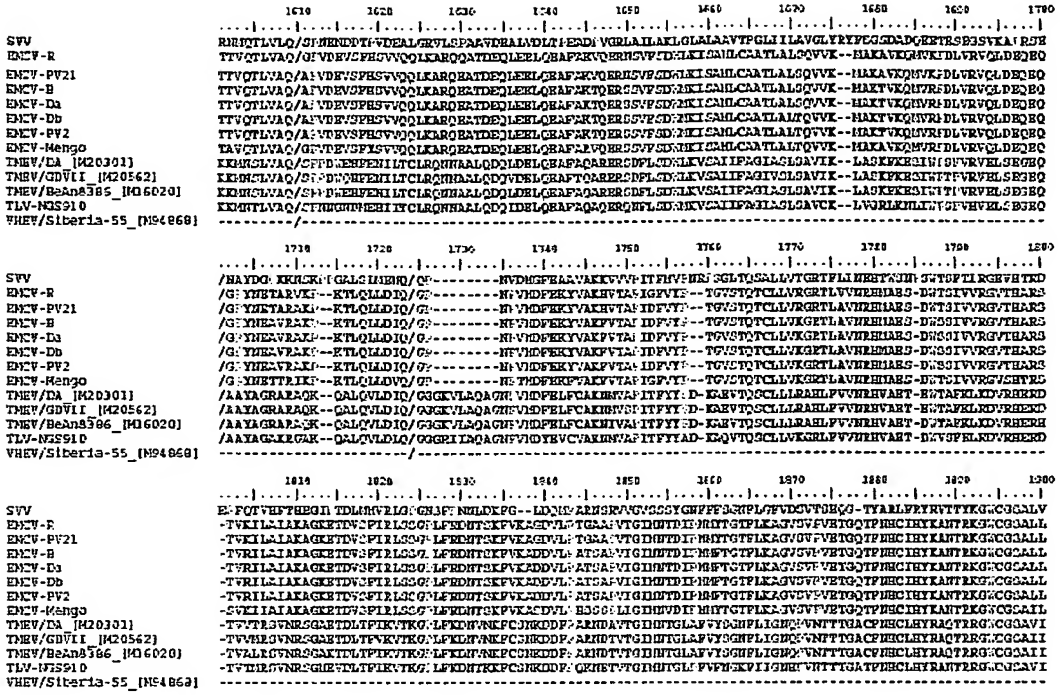
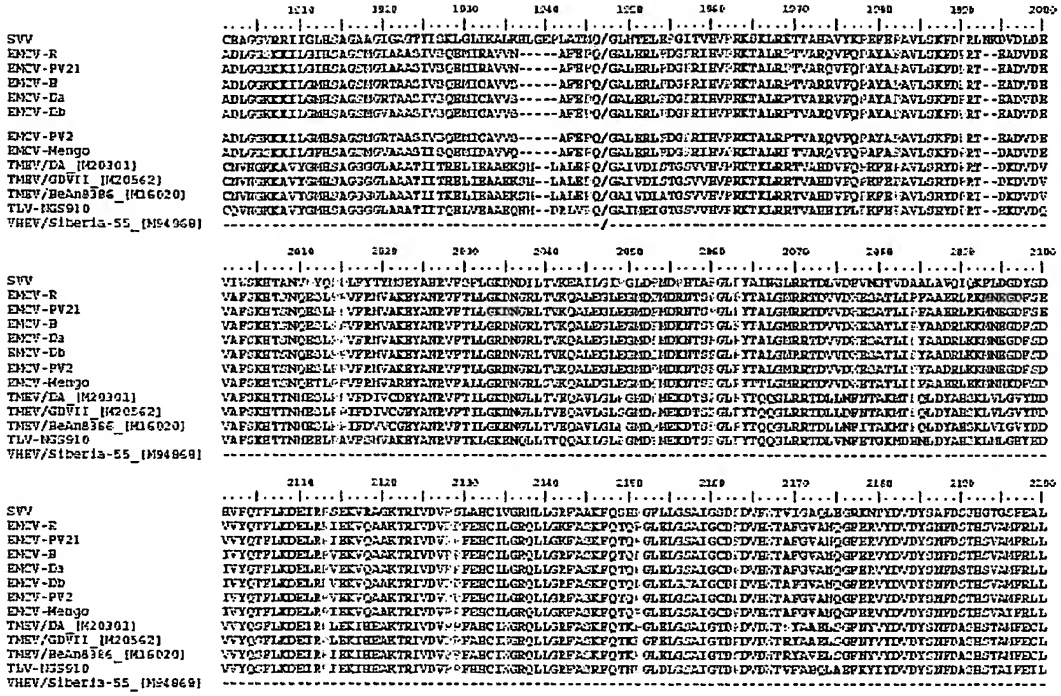


Fig. 28F


SVV
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ENCV-PV21
ENCV-B
ENCV-Da
ENCV-Db
ENCV-PV2
ENCV-Mengo
TMEV/DA_[M20301]
TMEV/GDVII_[M20562]
TMEV/BeAn8386_[M16020]
TLV-NSS910
VHEV/Siberia-55_[M94868]

Fig. 28G

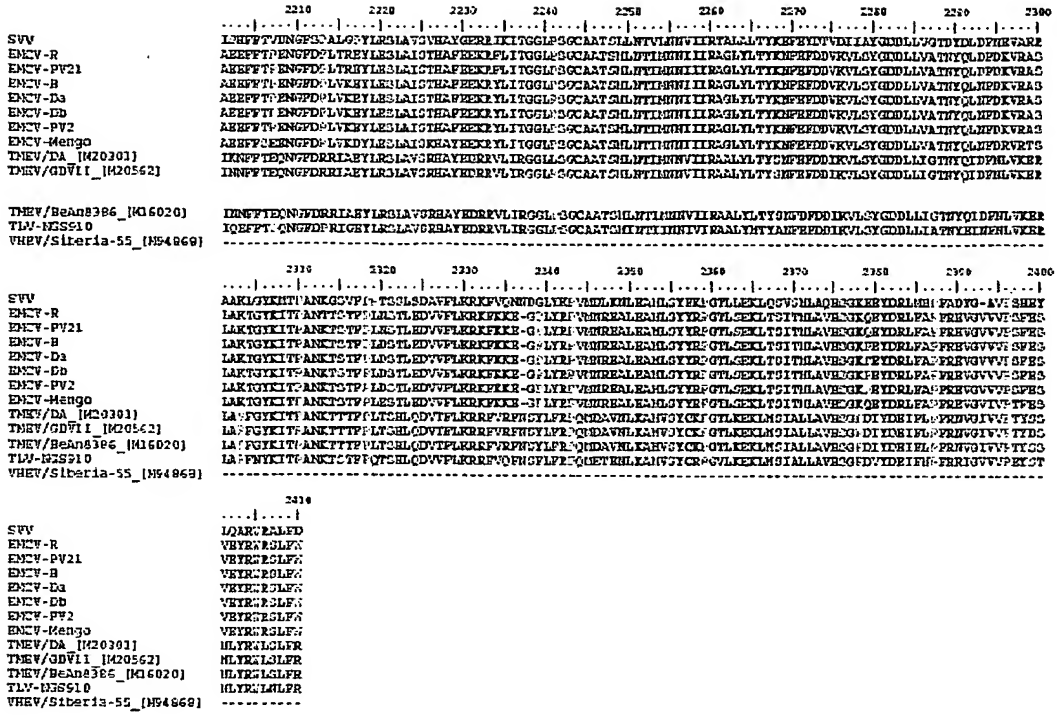


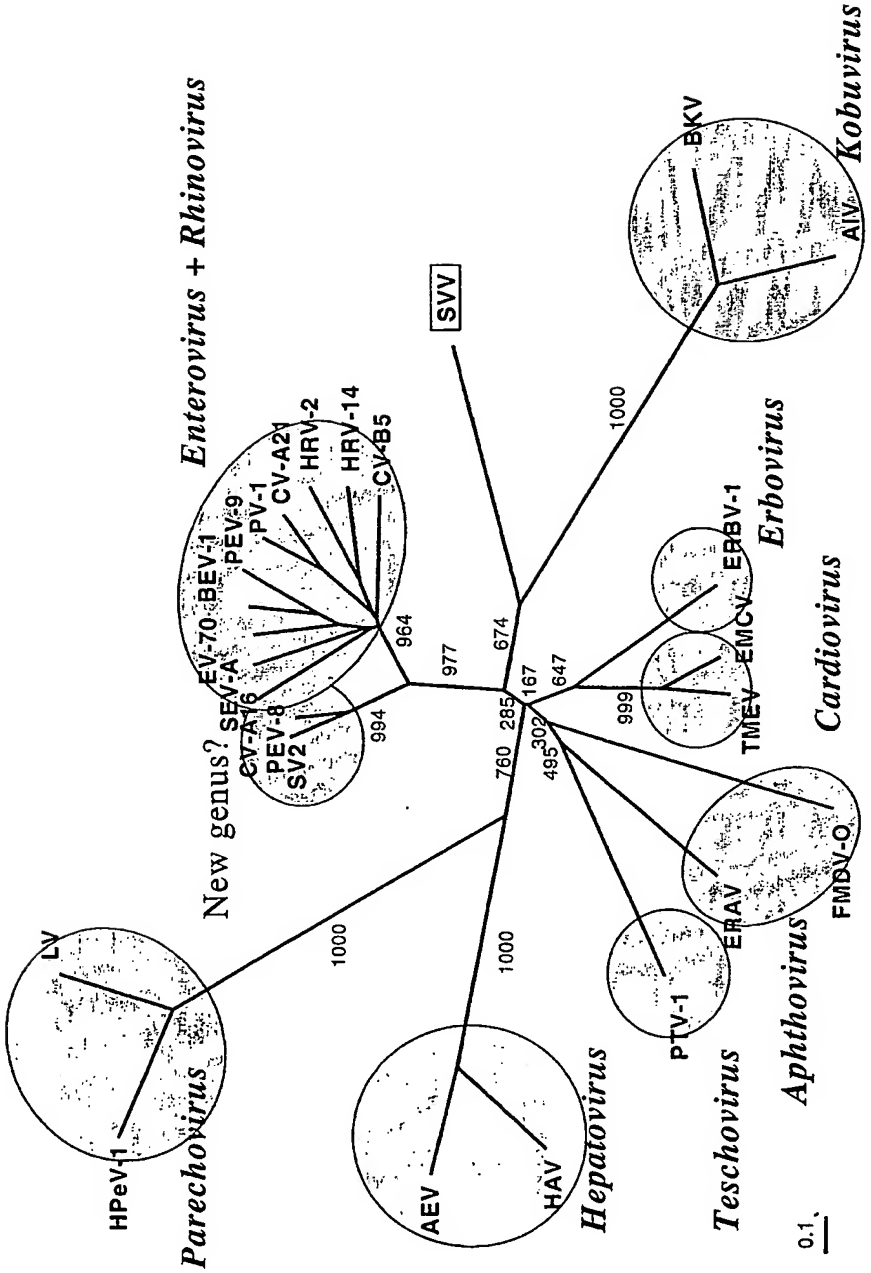
Fig. 28H

Polypeptide	aa	Predicted sequence
L Leader	?	No data
VP4 (1A)	?	No data
VP2 (1B)	>142	LAHHGNKKSLQELNEEQWVEMSDDYRTGKNMPFQSLGTYYRPPNWTWGPN FINPYQVTVFPHQILNARTSTSVDINVPYIGETPTQSSETQNSWTLLVMV LVPLDYKEGATTDPEITFSVRPTSPYFNGLRNRYTAGTDEEQ
VP3 (1C)	239	GPIPTAPRENSLMFLSTLPDDTVPAYGNVRTPPVNYLPGEITDLLQLARI PTLMAFERVPEPVPASDTYVPYVAVPTQFDDRPLISFPITLSDPVYQNTL VGAISSNFANYRGCIQITLTFCGPMMARGKFLLSYSPPNGTQPQTLSEAM QCTYSIWDIGLNSSWTFVVPYISPSDYRETRAITNSVYSADGWFSLHKLT KITLPPDCPQSPCILFFASAGEDYTLRLPVDCNPSYVFH
VP1 (1D)	259	STDNAETGVIEAGNTDTDFSGELAAPGPNHTNVKFLFDRSRLLNVIKVLE KDAVFPRPFPTQEGAQQDDGYFCLLTPRPTVASRPATRFGLYANPSGSGV LANTSLDFNFYSLACFTYFRSDLEVTVVSLEPDLEFAVGWFPSGSEYQAS SFVYDQLHVPFHFTGRTPRAFASKGGKVSFVLPWNSVSSVLPVRWGGASK LSSATRGLPAHADWGTIYAFVPRPNEKKSTAVKHVAVYIRYKNARAWCPS MLPFRSYKQ
2A	14	KMLMQSGDIETNPG
2B	128	PASDNPILEFLEAENDLVTLASLWKMVHSVQQTWRKYVKNDDFWPNLLSE LVGEGSVALAATLSNQASVKALLGLHFLSRGLNYTDFYSLLIEKCSSFFT VEPPPPPAENLMTKPSVKSKFRKLFKMQ
2C	322	GPMDKVKDWNQIAAGLKNFQFVRDLVKEVVDWLQAWINKEKASPVLQYQL EMKKLGPVALAHDAFMAGSGPPLSDDQIEYLQNLKSLALTLGKTNLAQSL TTMINAKQSSAQRVEPVVVVLRGKPGCGKGLASTLIAQAVSKRLYGSQSV YSLPPDPDFFDGYKGQFVTLMDDLGQNPDGQDFSTFCQMVSTAQFLPNMA DLAEKGRPFTSNLIIATTNLPHFSPVTIADPSAVSRRINYDLTLEVSEAY KKHTRLNFDLAFRRTDAPPIYPFAAHVPFVDVAVRFKNGHQNFNLLELVD SICTDIRAKQQGARNMQTLVLQ
3A	90	SPNENDDTPVDEALGRVLSPAAVDEALVDLTPEADPVGRLAILAKLGLAL AAVTPGLIILAVGLYRYFSGSDADQEETESEGSVKAPRSE
3B (VPg)	22	NAYDGPKKNSKPPGALSLMEMQ
3C (pro)	211	QPNVDMGFEAAVAKKVVVPITFMVPNRPSGLTQSALLVTGRTFLINEHTW SNPSWTSFTIRGEVHTRDEPFQTVHFTHHGIPTDLMMVRLGPGNSFPNNL DKFGLDQMPARNSRVVGVSSSYGNFFFSGNFLGFVDSVTSEQGTYARLFR YRVTTYKGWCGSALVCEAGGVRRIIGLHSAGAAGIGAGTYISKLGLIKAL KHLGEPLATMQ
3D (pol)	462	GLMTELEPGITVHVPRKSKLRKTTAHAVYKPEFEPAVLSKFDPRLNKDVD LDEVIWSKHTANVPYQPPLFYTYMSEYAHRVFSFLGKDNDILTVKEAILG IPGLDPMDPHTAPGLPYAINGLRRTDLVDFVNGTVDAALAVQIQKFLDGD YSDHVFQTFLKDEIRPSEKVRAGKTRIVDVPSLAHCIVGRMLLGRFAAKF QSHPGFLLGSAIGSDPDVFWTVIGAQLEGRKNTYDVDYSAFDSSHGTGSF EALISHFFTVDNGFSPALGPYLRSLAVSVHAYGERRIKITGGLPSGCAAT SLLNTVLNNVIIRTALALTYKEFEYDTVDIIAYGDDLLVGTDYDLDFNEV ARRAAKLGYKMTPANKGSVFPPTSSLSDAVFLKRKFVQNNDGLYKPVMDL KNLEAMLSYFKPGTLLEKLQSVSMLAQHSGKEEYDRLMHPFADYGAVPSH EYLQARWRALFD

Fig. 29

Genus	Species	Abbrev.	Serotype
Enterovirus	*Poliovirus*	PV-1	= Poliovirus 1
	Human enterovirus A	CV-A16	= Coxsackievirus A16
	Human enterovirus B	CV-B5	= Coxsackievirus B5
	Human enterovirus C	CV-A21	= Coxsackievirus A21
	Human enterovirus D	EV-70	= Enterovirus 70
	Simian enterovirus A	SEV-A	= Simian enterovirus A1
	Bovine enterovirus	BEV-1	= Bovine enterovirus 1
	Porcine enterovirus B	PEV-9	= Porcine enterovirus 9
Rhinovirus	*Human rhinovirus A*	HRV-2	= Human rhinovirus 2
	Human rhinovirus B	HRV-14	= Human rhinovirus 14
New genus?	Not yet designated	SV2	= Simian virus 2
	Porcine enterovirus A	PEV-8	= Porcine enterovirus 8
Cardiovirus	*Encephalomyocarditis virus*	EMCV	= Encephalomyocarditis virus
	Theilovirus	TMEV	= Theiler's murine encephalomyelitis virus
	?	SVV	= Seneca Valley virus
Aphthovirus	*Foot-and-mouth disease virus*	FMDV-O	= Foot-and-mouth disease virus O
	Equine rhinitis A virus	ERAV	= Equine rhinitis A virus
Hepatovirus	*Hepatitis A virus*	HAV	= Hepatitis A virus
	Avian encephalomyelitis-like viruses	AEV	= Avian encephalomyelitis virus
Parechovirus	*Human parechovirus*	HPeV-1	= Human parechovirus 1
	Ljungan virus	LV	= Ljungan virus
Kobuvirus	*Aichi virus*	AiV	= Aichi virus
	Bovine kobuvirus	BKV	= Bovine kobuvirus
Erbovirus	*Equine rhinitis B virus*	ERBV-1	= Equine rhinitis B virus 1
Teschovirus	*Porcine teschovirus*	PTV-1	= Porcine teschovirus 1

Fig. 30 (35/99)


Enterovirus + Rhinovirus
New genus?
Parechovirus
Hepatovirus
Teschovirus
Aphthovirus
Cardiovirus
Erbovirus
Kobuvirus
HPeV-1
LV
AEV
HAV
PTV-1
ERAV
FMDV-O
TMEV
EMCV
EBBV-1
SVV
EV-70
BEV-1
PEV-9
PV-1
CV-A21
HRV-2
HRV-14
CV-B5
SEV-A
CV-A16
PEV-8
SV2
BKV
AiV
1000
1000
1000
994
964
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674
760
285
167
647
495
302
999
0.1

Fig. 31 (36/99)

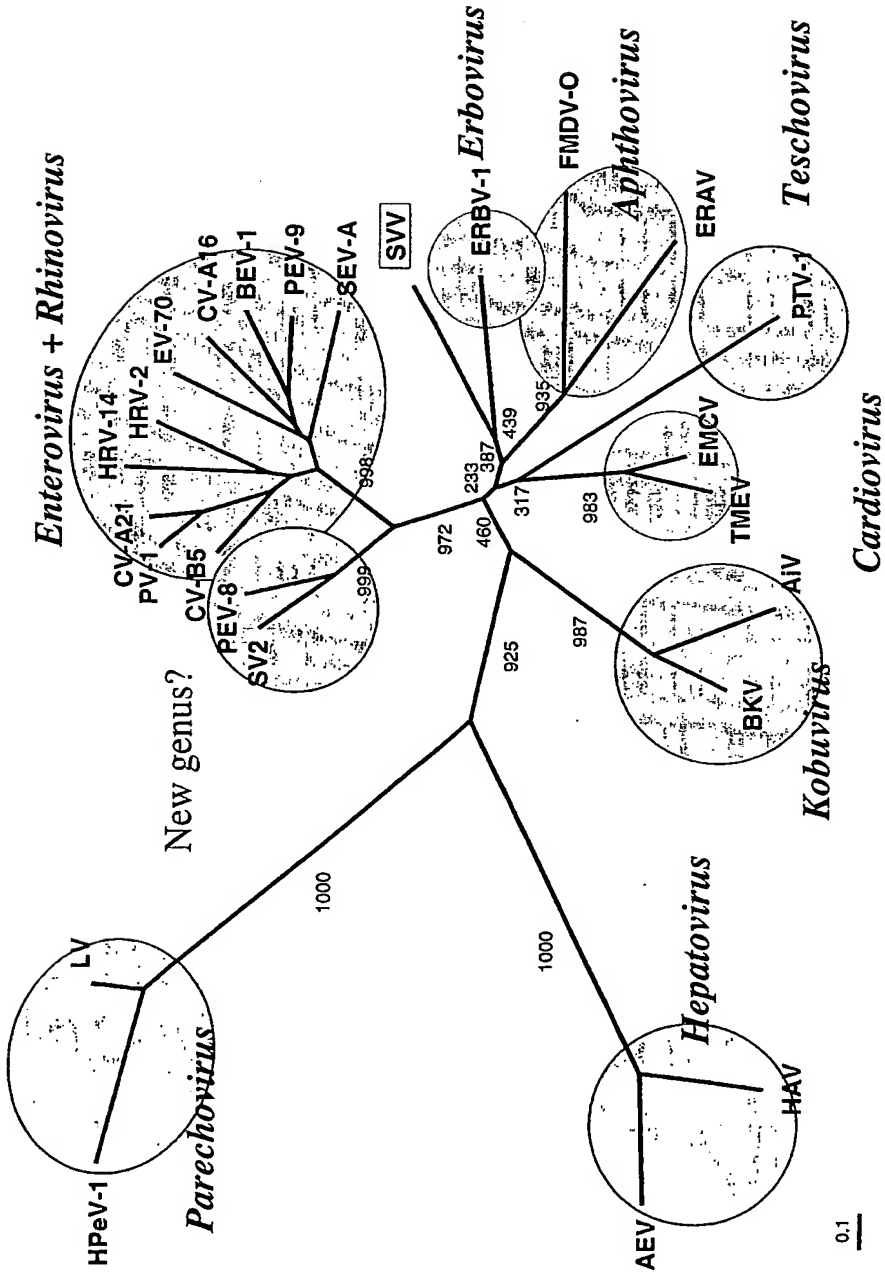
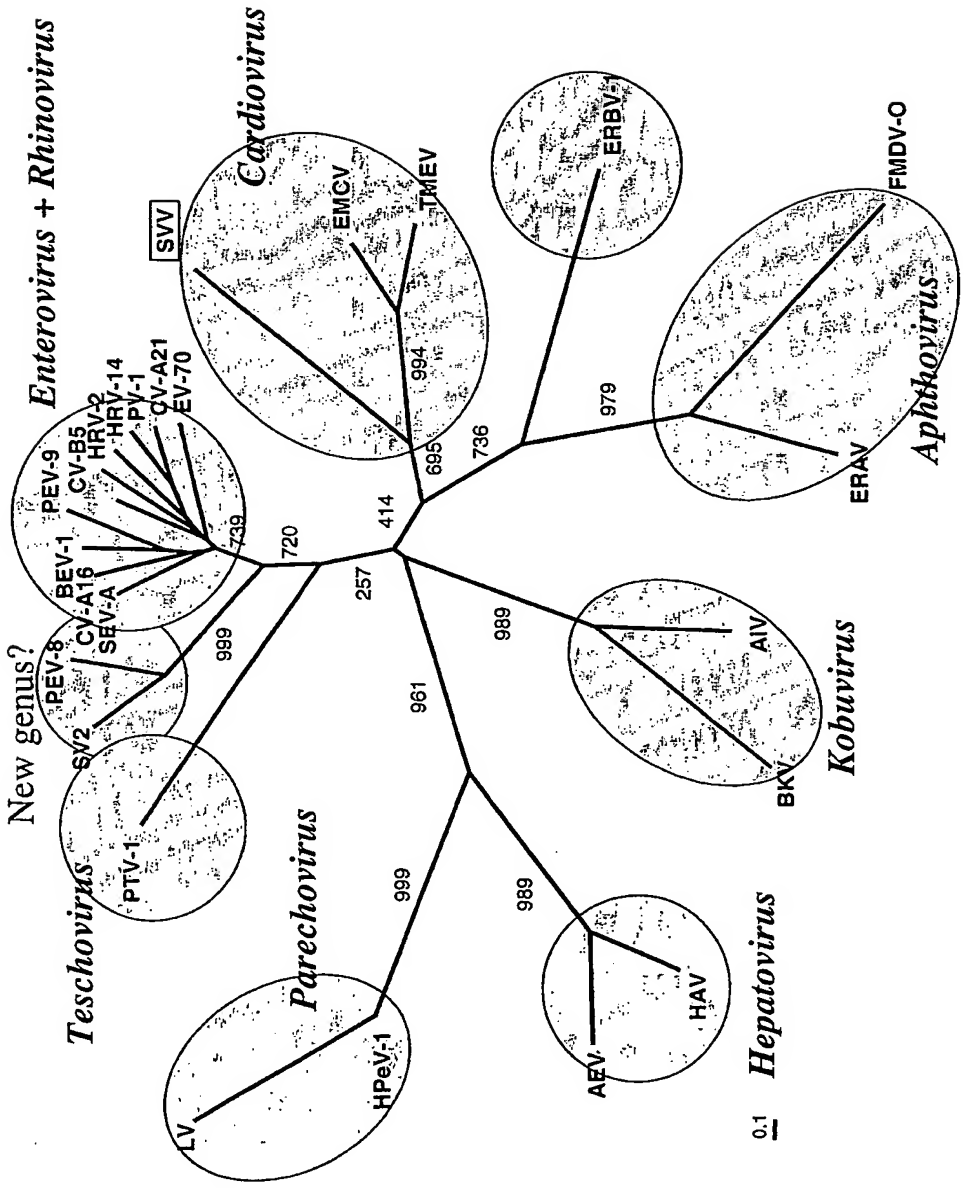

Enterovirus + Rhinovirus
HRV-14
HRV-2
EV-70
CV-A16
BEV-1
PEV-9
SEV-A
CV-A21
PV-1
CV-B5
PEV-8
SV2
998
999
SVV
ERBV-1
Erbovirus
FMDV-O
Aphthovirus
ERAV
Teschovirus
PTV-1
EMCV
TMEV
AiV
BKV
Kobuvirus
Cardiovirus
New genus?
HPeV-1
LV
Parechovirus
AEV
HAV
Hepatovirus
1000
1000
925
987
972
460
233
387
439
935
317
983
0.1

Fig. 32 (37/99)


Enterovirus + Rhinovirus
Cardiovirus
Aphthovirus
Kobuvirus
Parechovirus
Hepatovirus
Teschovirus
New genus?
SVV
EMCV
TMEV
ERBV
FMDV-O
ERAV
AIV
BKV
HPeV-1
LV
AEV
HAV
PTV-1
SV2
PEV-8
BEV-1
CV-A16
SEV-A
PEV-9
CV-B5
HRV-2
HRV-14
PV-1
CV-A21
EV-70
739
720
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414
695
994
736
979
989
961
999
999
989
0.1

Fig. 33 (38/99)

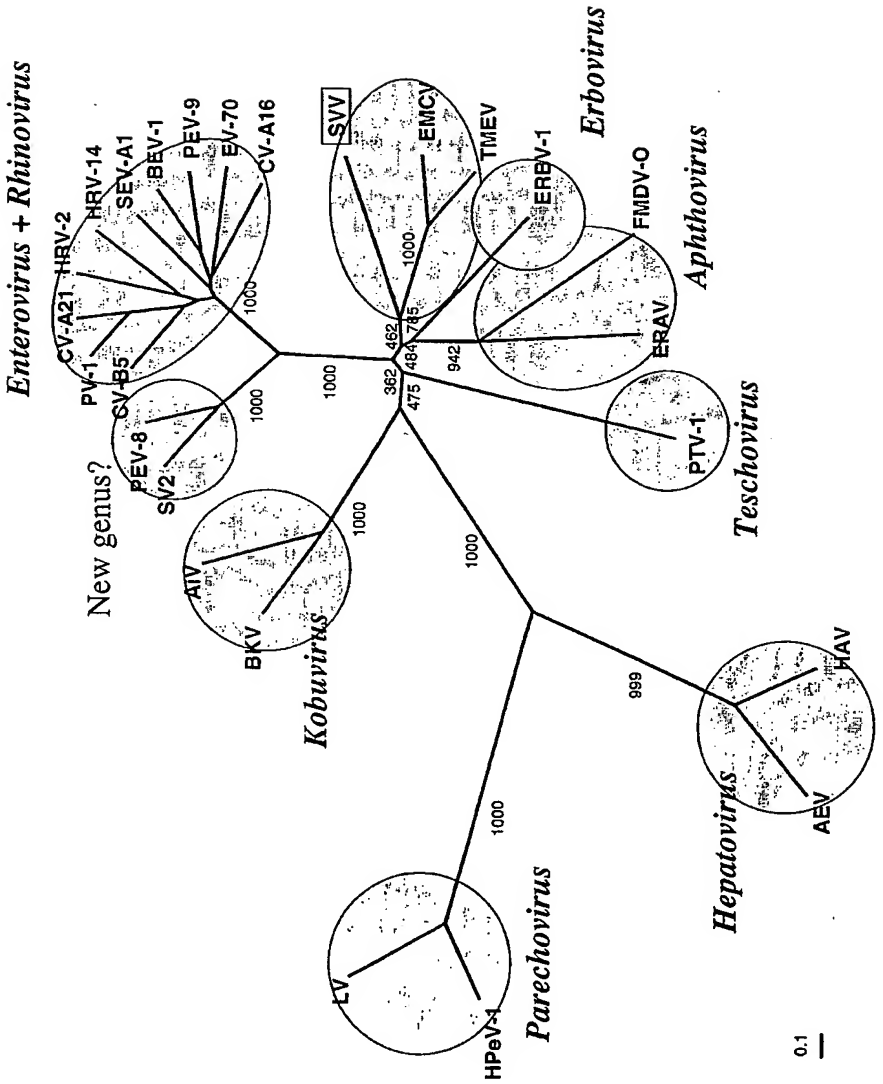
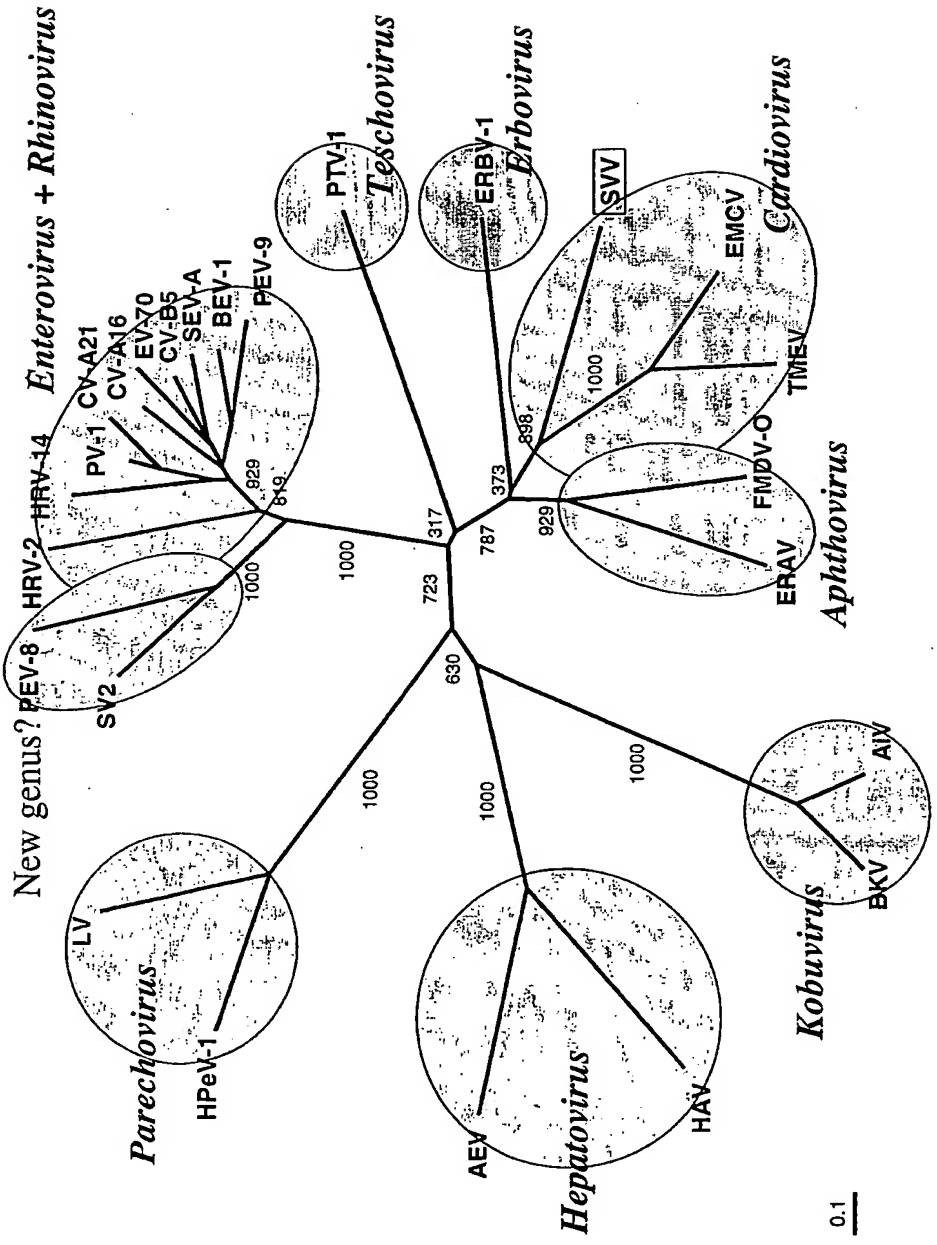

Enterovirus + Rhinovirus
CV-A21
HRV-2
HRV-14
SEV-A1
BEV-1
PEV-9
EV-70
CV-A16
PV-1
CV-B5
PEV-8
SV2
New genus?
AIV
BKV
Kobuvirus
SVV
EMCV
TMEV
ERBV-1
Erbovirus
FMDV-O
ERAV
Aphthovirus
PTV-1
Teschovirus
LV
HPeV-1
Parechovirus
Hepatovirus
AEV
HAV
1000
999
942
484
462
785
475
362
0.1

Fig. 34 (39/99)


New genus?
PEV-8
SV2
HRV-2
HRV-14
PV-1
CV-A21
CV-A16
EV-70
CV-B5
SEV-A
BEV-1
PEV-9
Enterovirus + Rhinovirus
PTV-1
Teschovirus
ERBV-1
Erbovirus
SVV
EMCV
TMEV
FMDV-O
ERAV
Cardiovirus
Aphthovirus
LV
HPeV-1
Parechovirus
AEV
HAV
Hepatovirus
AiV
BKV
Kobuvirus
1000
929
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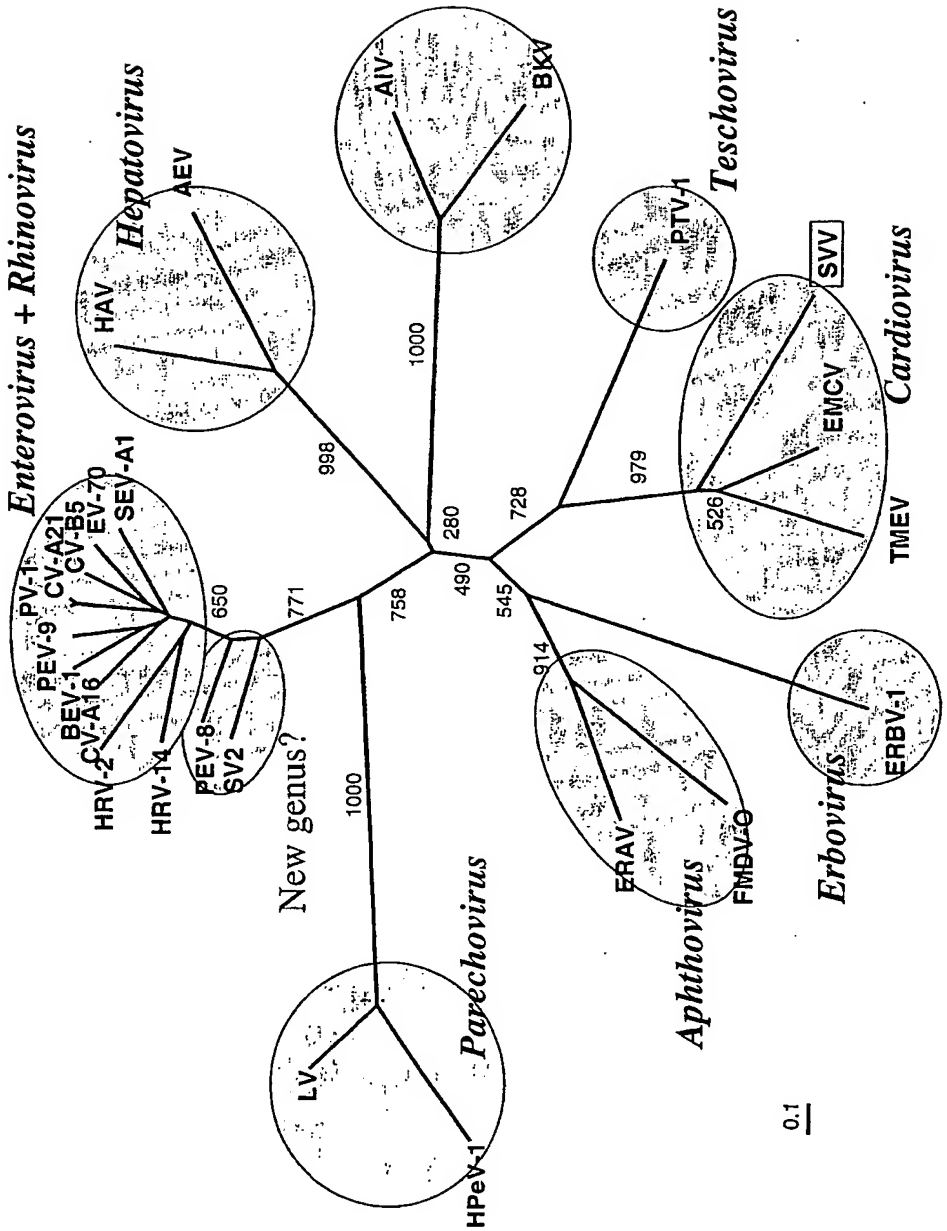
373
898
1000
929
630
1000
1000
1000
0.1

FIG. 35 (40/99)


Enterovirus + Rhinovirus
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CV-B5
EV-70
SEV-A1
PEV-9
BEV-1
CV-A16
HRV-2
HRV-14
PEV-8
SV2
Hepatovirus
HAV
AEV
AIV
BKV
PTV-1
Teschovirus
SVV
EMCV
Cardiovirus
TMEV
ERBV-1
Erbovirus
ERAV
FMDV-O
Aphthovirus
LV
HPeV-1
Parechovirus
New genus?
998
1000
280
728
979
526
490
545
914
771
650
758
1000
0.1

FIG. 36 (41/99)

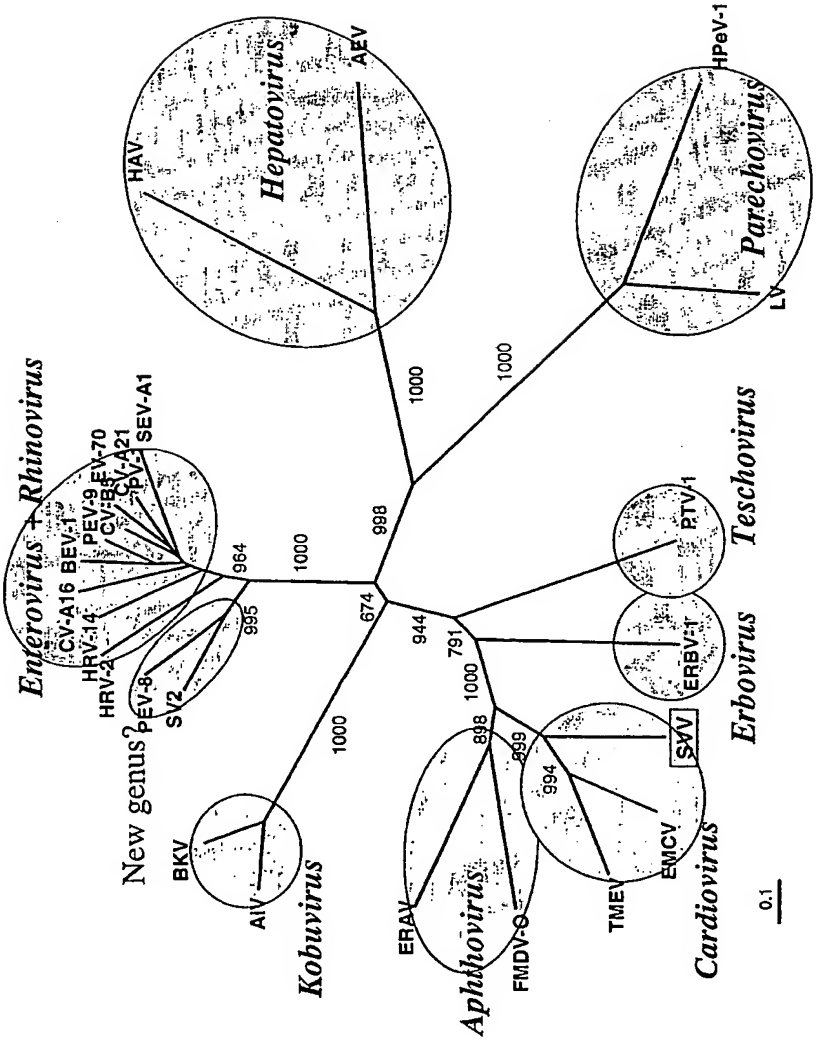

Enterovirus + Rhinovirus
CV-A16
BEV-1
PEV-9
EV-70
CV-B5
CV-A21
PV-3
SEV-A1
HRV-14
HRV-2
PEV-8
SV2
964
995
1000
674
998
Hepatovirus
HAV
AEV
1000
1000
Parechovirus
HPeV-1
LV
New genus?
BKV
AIV
Kobuvirus
1000
944
791
1000
PTV-1
ERBV-1
Teschovirus
Erbovirus
ERAV
898
FMDV-O
TMEV
999
994
SVV
EMCV
Cardiovirus
Aphthovirus
0.1

FIG. 37 (42/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	33	35	24	26	30	24	24	24	29	29	30	35	25	27	26	25	31	31	27	21	20	19	18	18
EMCV	33	100	70	32	35	47	29	32	30	31	34	30	34	34	31	34	33	40	37	20	21	25	25	22	21
TMEV	35	70	100	33	34	45	31	35	34	30	32	31	34	33	31	31	28	36	37	21	21	25	25	23	21
FMDV-O	24	32	33	100	31	35	31	27	26	30	27	25	28	28	25	31	25	32	29	15	17	24	24	19	17
ERAV	26	35	34	31	100	35	36	32	31	28	35	30	31	28	27	29	30	39	34	22	22	20	24	18	22
ERBV	30	47	45	35	35	100	29	26	28	34	31	31	33	30	31	33	32	36	35	21	21	26	28	20	21
PTV-1	24	29	31	31	36	29	100	30	31	27	29	26	30	30	30	30	26	28	30	18	22	25	22	19	22
PV-1	24	32	35	27	32	26	30	100	71	52	52	51	55	54	48	53	51	42	44	21	23	19	21	18	20
CV-A21	24	30	34	26	31	28	31	71	100	51	53	52	52	53	47	54	55	43	46	19	23	23	22	20	19
CV-A16	29	31	30	30	28	34	27	52	51	100	52	53	53	54	52	52	48	49	48	18	19	22	22	19	17
CV-B5	29	34	32	27	35	31	29	52	53	52	100	55	54	53	49	56	51	51	48	20	17	23	22	20	18
EV-70	30	30	31	25	30	31	26	51	52	53	55	100	57	63	62	52	53	46	49	20	20	25	23	18	17
SEV-A	35	34	34	28	31	33	30	55	52	53	54	57	100	55	54	47	50	43	45	21	20	21	22	22	19
BEV-1	25	34	33	28	28	30	30	54	53	54	53	63	55	100	67	54	52	45	46	20	23	21	21	16	16
PEV-9	27	31	31	25	27	31	30	48	47	52	49	62	54	67	100	53	49	45	45	21	23	24	25	20	18
HRV-14	26	34	31	31	29	33	30	53	54	52	56	52	47	54	53	100	61	46	49	23	21	22	21	19	21
HRV-2	25	33	28	25	30	32	26	51	55	48	51	53	50	52	49	61	100	46	46	22	21	22	24	20	22
SV2	31	40	36	32	39	36	28	42	43	49	51	46	43	45	45	46	46	100	74	23	22	25	27	20	19
PEV-8	31	37	37	29	34	35	30	44	46	48	48	49	45	46	45	49	46	74	100	24	22	25	26	18	21
AIV	27	20	21	15	22	21	18	21	19	18	20	20	21	20	21	23	22	23	24	100	56	19	18	16	16
BKV	21	21	21	17	22	21	22	23	23	19	17	20	20	23	23	21	21	22	22	56	100	16	18	16	16
HAV	20	25	25	24	20	26	25	19	23	22	23	25	21	21	24	22	22	25	25	19	16	100	57	18	23
AEV	19	25	25	24	24	28	22	21	22	22	22	23	22	21	25	21	24	27	26	18	18	57	100	18	23
HPeV-1	18	22	23	19	18	20	19	18	20	19	20	18	22	16	20	19	20	20	18	16	16	18	18	100	53
LV	18	21	21	17	22	21	22	20	19	17	18	17	19	16	18	21	22	19	21	16	16	23	23	53	100

Fig. 38 (43/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	42	39	32	34	43	31	32	30	25	29	26	30	25	29	28	28	35	30	26	28	19	17	17	19
EMCV	42	100	67	29	38	39	35	28	31	29	31	31	29	31	30	26	31	31	35	27	26	19	18	17	18
TMEV	39	67	100	31	37	35	33	29	29	31	29	27	27	27	28	26	30	31	35	27	28	18	17	17	20
FMDV-O	32	29	31	100	39	31	23	26	23	26	27	25	26	25	24	26	23	30	32	20	25	14	15	14	17
ERAV	34	38	37	39	100	38	25	25	24	20	27	23	23	23	25	24	27	31	32	25	24	20	15	15	19
ERBV	43	39	35	31	38	100	30	27	29	27	28	27	30	29	28	28	28	34	34	27	29	21	19	18	17
PTV-1	31	35	33	23	25	30	100	23	22	24	24	23	27	25	28	22	27	28	27	24	23	16	16	19	18
PV-1	32	28	29	26	25	27	23	100	75	42	55	42	46	41	43	43	50	36	36	24	29	17	17	15	15
CV-A21	30	31	29	23	24	29	22	75	100	43	61	42	46	42	45	48	53	36	35	24	26	16	20	13	13
CV-A16	25	29	31	26	20	27	24	42	43	100	48	51	50	55	54	40	41	40	38	24	26	13	14	13	14
CV-B5	29	31	29	27	27	28	24	55	61	48	100	47	48	49	49	51	52	40	36	24	27	16	17	14	15
EV-70	26	31	27	25	23	27	23	42	42	51	47	100	49	49	53	45	44	32	38	25	27	16	15	15	15
SEV-A	30	29	27	26	23	30	27	46	46	50	48	49	100	48	53	41	43	37	37	28	28	13	14	14	15
BEV-1	25	31	27	25	23	29	25	41	42	55	49	49	48	100	63	45	45	40	40	23	25	11	12	13	17
PEV-9	29	30	28	24	25	28	28	43	45	54	49	53	53	63	100	43	48	39	39	22	27	15	12	14	13
HRV-14	28	26	26	26	24	28	22	43	48	40	51	45	41	45	43	100	52	33	33	26	27	16	17	14	19
HRV-2	28	31	30	23	27	28	27	50	53	41	52	44	43	45	48	52	100	35	38	25	28	19	16	14	17
SV2	35	31	31	30	31	34	28	36	36	40	40	32	37	40	39	33	35	100	63	25	29	18	16	16	21
PEV-8	30	35	35	32	32	34	27	36	35	38	36	38	37	40	39	33	38	63	100	24	30	18	17	18	18
AIV	26	27	27	20	25	27	24	24	24	24	24	25	28	23	22	26	25	25	24	100	58	16	18	17	19
BKV	28	26	28	25	24	29	23	29	26	26	27	27	28	25	27	27	28	29	30	58	100	17	20	16	19
HAV	19	19	18	14	20	21	16	17	16	13	16	16	13	11	15	16	19	18	18	16	17	100	53	18	19
AEV	17	18	17	15	15	19	16	17	20	14	17	15	14	12	12	17	16	16	17	18	20	53	100	20	20
HPeV-1	17	17	17	14	15	18	19	15	13	13	14	15	14	13	14	14	14	16	18	17	16	18	20	100	55
LV	19	18	20	17	19	17	18	15	13	14	15	15	15	17	13	19	17	21	18	19	19	19	20	55	100

Fig. 39 (44/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AiV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	20	21	12	13	13	11	17	16	16	16	14	17	13	13	18	16	14	13	13	13	9	10	11	8
EMCV	20	100	48	11	15	16	15	16	16	14	14	15	18	17	17	17	15	16	16	14	13	12	12	11	11
TMEV	21	48	100	12	15	15	14	15	16	16	13	13	17	14	15	16	15	16	15	17	14	15	13	9	11
FMDV-O	12	11	12	100	23	13	9	11	13	12	11	11	11	10	11	10	13	9	12	12	8	11	10	11	10
ERAV	13	15	15	23	100	18	12	13	14	16	14	11	14	14	12	14	14	10	14	11	9	9	9	9	11
ERBV	13	16	15	13	18	100	13	13	13	14	15	15	13	12	11	12	12	12	13	17	12	10	12	13	11
PTV-1	11	15	14	9	12	13	100	19	18	21	19	19	18	17	17	18	17	17	17	16	12	12	9	13	12
PV-1	17	16	15	11	13	13	19	100	57	34	46	34	41	35	32	46	36	23	21	16	16	10	10	14	10
CV-A21	16	16	16	13	14	13	18	57	100	36	43	37	39	33	35	42	40	24	24	15	13	10	14	14	11
CV-A16	16	14	16	12	16	14	21	34	36	100	37	38	42	43	40	35	37	27	24	15	15	12	13	13	8
CV-B5	16	14	13	11	14	15	19	46	43	37	100	42	40	38	32	38	42	27	25	14	16	13	12	13	9
EV-70	14	15	13	11	11	15	19	34	37	38	42	100	37	39	33	38	37	24	23	13	13	16	12	13	12
SEV-A	17	18	17	11	14	13	18	41	39	42	40	37	100	39	40	39	40	25	25	13	13	13	13	14	12
BEV-1	13	17	14	10	14	12	17	35	33	43	38	39	39	100	46	37	35	25	25	15	14	15	11	12	10
PEV-9	13	17	15	11	12	11	17	32	35	40	32	33	40	46	100	36	34	23	22	13	13	12	11	12	9
HRV-14	18	17	16	10	14	12	18	46	42	35	38	38	39	37	36	100	42	23	22	15	16	11	13	14	10
HRV-2	16	15	15	13	14	12	17	36	40	37	42	37	40	35	34	42	100	24	21	16	14	13	14	14	9
SV2	14	16	16	9	10	12	17	23	24	27	27	24	25	25	23	23	24	100	46	15	12	11	12	12	11
PEV-8	13	16	15	12	14	13	17	21	24	24	25	23	25	25	22	22	21	46	100	14	12	13	12	13	8
AiV	13	14	17	12	11	17	16	16	15	15	14	13	13	15	13	15	16	15	14	100	28	11	13	11	10
BKV	13	13	14	8	9	12	12	16	13	15	16	13	13	14	13	16	14	12	12	28	100	11	11	13	10
HAV	9	12	15	11	9	10	12	10	10	12	13	16	13	15	12	11	13	11	13	11	11	100	43	15	14
AEV	10	12	13	10	9	12	9	10	14	13	12	12	13	11	11	13	14	12	12	13	11	43	100	18	14
HPeV-1	11	11	9	11	9	13	13	14	14	13	13	13	14	12	12	14	14	12	13	11	13	15	18	100	43
LV	8	11	11	10	11	11	12	10	11	8	9	12	12	10	9	10	9	11	8	10	10	14	14	43	100

Fig. 40 (45/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AiV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	34	33	26	27	30	22	23	23	22	24	22	24	21	22	22	21	25	24	22	22	15	15	14	12
EMCV	34	100	59	25	28	31	25	22	21	22	24	22	23	23	22	21	23	24	25	21	21	15	16	15	14
TMEV	33	59	100	27	29	30	24	23	22	24	21	21	22	22	22	22	22	24	26	24	24	15	15	14	14
FMDV-O	26	25	27	100	33	27	20	21	20	21	20	20	22	19	18	22	19	22	23	17	19	13	12	13	12
ERAV	27	28	29	33	100	32	23	21	20	17	23	19	20	20	20	21	21	25	25	22	20	16	15	13	15
ERBV	30	31	30	27	32	100	24	22	23	23	24	23	24	23	22	22	21	26	26	24	21	16	16	15	13
PTV-1	22	25	24	20	23	24	100	19	20	20	19	20	22	19	22	19	19	20	20	20	20	15	13	13	14
PV-1	23	22	23	21	21	22	19	100	66	39	50	40	44	40	39	44	43	29	30	18	22	14	14	11	11
CV-A21	23	21	22	20	20	23	20	66	100	40	51	42	42	40	40	44	44	29	32	18	20	14	15	10	11
CV-A16	22	22	24	21	17	23	20	39	40	100	43	47	46	49	46	40	40	33	32	19	20	12	11	11	11
CV-B5	24	24	21	20	23	24	19	50	51	43	100	46	44	44	41	45	46	35	35	19	20	14	14	12	10
EV-70	22	22	21	20	19	23	20	40	42	47	46	100	45	47	45	42	41	30	32	20	20	14	12	13	11
SEV-A	24	23	22	22	20	24	22	44	42	46	44	45	100	46	48	41	41	32	33	21	21	12	12	12	11
BEV-1	21	23	22	19	20	23	19	40	40	49	44	47	46	100	56	42	42	33	33	19	20	11	11	11	13
PEV-9	22	22	22	18	20	22	22	39	40	46	41	45	48	56	100	41	42	32	32	18	20	12	10	12	10
HRV-14	22	21	22	22	21	22	19	44	44	40	45	42	41	42	41	100	47	30	29	20	20	14	14	12	13
HRV-2	21	23	22	19	21	21	19	43	44	40	46	41	41	42	42	47	100	30	30	20	20	14	12	11	12
SV2	25	24	24	22	25	26	20	29	29	33	35	30	32	33	32	30	30	100	57	19	21	15	14	14	14
PEV-8	24	25	26	23	25	26	20	30	32	32	35	32	33	33	32	29	30	57	100	18	22	16	15	14	13
AiV	22	21	24	17	22	24	20	18	18	19	19	20	21	19	18	20	20	19	18	100	46	14	14	14	14
BKV	22	21	24	19	20	21	20	22	20	20	20	20	21	20	20	20	20	21	22	46	100	13	15	13	13
HAV	15	15	15	13	16	16	15	14	14	12	14	14	12	11	12	14	14	15	16	14	13	100	49	16	15
AEV	15	16	15	12	15	16	13	14	15	11	14	12	12	11	10	14	12	14	15	14	15	49	100	18	17
HPeV-1	14	15	14	13	13	15	13	11	10	11	12	13	12	11	12	12	11	14	14	14	13	16	18	100	50
LV	12	14	14	12	15	13	14	11	11	11	10	11	11	13	10	13	12	14	13	14	13	15	17	50	100

* not including VP4 or part of VP2

Fig. 41 (46/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AiV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	41	39	35	34	35	32	29	28	30	29	30	26	26	26	29	27	28	29	26	23	25	26	21	20
EMCV	41	100	60	37	36	35	30	27	27	29	28	28	27	27	27	25	27	26	25	23	24	27	25	20	21
TMEV	39	60	100	36	38	35	31	28	26	26	29	28	26	25	24	27	26	29	25	23	23	26	25	20	21
FMDV-O	35	37	36	100	47	38	30	31	28	32	30	32	30	31	27	31	28	31	31	27	28	25	24	20	21
ERAV	34	36	38	47	100	37	35	31	28	27	29	31	28	28	27	27	28	28	30	25	25	24	24	22	20
ERBV	35	35	35	38	37	100	27	29	27	30	30	29	30	27	30	28	26	27	27	23	23	27	24	23	24
PTV-1	32	30	31	30	35	27	100	27	28	30	30	29	28	29	27	27	29	28	28	27	26	21	22	21	24
PV-1	29	27	28	31	31	29	27	100	79	64	63	62	58	59	58	61	49	45	39	24	22	20	23	24	23
CV-A21	28	27	26	28	28	27	28	79	100	58	59	56	55	56	56	56	43	41	38	24	21	19	22	23	21
CV-A16	30	29	26	32	27	30	30	64	58	100	66	60	63	64	58	55	48	44	40	24	23	22	24	24	24
CV-B5	29	28	29	30	29	30	30	63	59	66	100	67	71	63	59	57	46	43	37	23	23	22	24	26	25
EV-70	30	28	28	32	31	29	29	62	56	60	67	100	65	59	58	53	45	43	39	24	23	21	22	25	23
SEV-A	26	27	26	30	28	30	28	58	55	63	71	65	100	61	60	54	45	42	40	21	22	21	22	25	25
BEV-1	26	27	25	31	28	27	29	59	56	64	63	59	61	100	67	53	47	44	40	23	23	21	21	24	24
PEV-9	26	27	24	27	27	30	27	58	56	58	59	56	60	67	100	52	42	41	38	22	21	22	22	23	21
HRV-14	29	25	27	31	27	28	27	61	56	55	57	53	54	53	52	100	47	42	40	21	20	21	19	22	23
HRV-2	27	27	26	28	28	26	29	49	43	48	46	45	45	47	42	47	100	40	37	22	21	21	20	22	24
SV2	28	26	29	31	28	27	28	45	41	44	43	43	42	44	41	42	40	100	52	22	22	22	20	21	21
PEV-8	29	25	25	31	30	27	28	39	38	40	37	39	40	40	38	40	37	52	100	21	20	21	20	21	20
AiV	26	23	23	27	25	23	27	24	24	24	23	24	21	23	22	21	22	22	21	100	69	24	22	22	22
BKV	23	24	23	28	25	23	26	22	21	23	23	23	22	23	21	20	21	22	20	69	100	25	23	22	22
HAV	25	27	26	25	24	27	21	20	19	22	22	21	21	21	22	21	21	22	21	24	25	100	41	20	21
AEV	26	25	25	24	24	24	22	23	22	24	24	22	22	21	22	19	20	20	20	22	23	41	100	22	23
HPeV-1	21	20	20	20	22	23	21	24	23	24	26	25	25	24	23	22	22	21	21	22	22	20	22	100	50
LV	20	21	21	21	20	24	24	23	21	24	25	23	25	24	21	23	24	21	20	22	22	21	23	50	100

Fig. 42 (47/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	38	37	24	21	19	27	19	18	14	16	17	14	18	19	18	15	17	16	16	14	17	16	13	14
EMCV	38	100	48	27	27	18	24	18	18	18	20	20	19	17	20	19	20	19	19	15	16	22	18	17	19
TMEV	37	48	100	20	28	20	25	19	20	20	18	20	16	19	18	15	18	17	17	16	17	17	16	16	19
FMDV-O	24	27	20	100	40	23	20	19	19	22	17	19	17	22	23	18	20	20	20	16	13	20	20	13	15
ERAV	21	27	28	40	100	25	22	21	20	20	20	21	22	20	18	19	20	21	22	19	20	22	22	15	17
ERBV	19	18	20	23	25	100	18	19	20	16	17	17	17	20	16	18	14	20	17	18	20	15	20	14	16
PTV-1	27	24	25	20	22	18	100	20	19	20	19	20	19	20	20	22	21	18	21	16	17	20	15	13	14
PV-1	19	18	19	19	21	19	20	100	95	55	60	60	56	57	60	46	44	43	47	21	17	18	18	18	18
CV-A21	18	18	20	19	20	20	19	95	100	56	59	57	57	57	60	46	44	42	46	22	17	18	18	19	18
CV-A16	14	18	20	22	20	16	20	55	56	100	56	54	57	57	60	51	45	46	50	17	18	19	18	20	22
CV-B5	16	20	18	17	20	17	19	60	59	56	100	64	55	58	56	52	49	46	52	20	19	19	19	19	20
EV-70	17	20	20	19	21	17	20	60	57	54	64	100	57	54	55	51	48	43	51	20	20	21	18	14	17
SEV-A	14	19	16	17	22	17	19	56	57	57	55	57	100	54	55	53	46	40	49	20	17	19	18	17	19
BEV-1	18	17	19	22	20	20	20	57	57	57	58	54	54	100	63	47	47	41	44	16	16	20	20	19	16
PEV-9	19	20	18	23	18	16	20	60	60	60	56	55	55	63	100	50	45	45	50	18	14	19	18	18	21
HRV-14	18	19	15	18	19	18	22	46	46	51	52	51	53	47	50	100	50	42	49	20	20	20	20	16	18
HRV-2	15	20	18	20	20	14	21	44	44	45	49	48	46	47	45	50	100	43	43	20	23	18	19	17	18
SV2	17	19	17	20	21	20	18	43	42	46	46	43	40	41	45	42	43	100	60	16	18	18	19	22	24
PEV-8	16	19	17	20	22	17	21	47	46	50	52	51	49	44	50	49	43	60	100	16	17	19	20	20	19
AIV	16	15	16	16	19	18	16	21	22	17	20	20	20	16	18	20	20	16	16	100	48	19	17	13	15
BKV	14	16	17	13	20	20	17	17	17	18	19	20	17	16	14	20	23	18	17	48	100	18	18	13	14
HAV	17	22	17	20	22	15	20	18	18	19	19	21	19	20	19	20	18	18	19	19	18	100	40	16	15
AEV	16	18	16	20	22	20	15	18	18	18	19	18	18	20	18	20	19	19	20	17	18	40	100	14	16
HPeV-1	13	17	16	13	15	14	13	18	19	20	19	14	17	19	18	16	17	22	20	13	13	16	14	100	48
LV	14	19	19	15	17	16	14	18	18	22	20	17	19	16	21	18	18	24	19	15	14	15	16	48	100

Fig. 43 (48/99)

	Cardiovirus			Aphthovirus		Erbo	Tescho	Enterovirus								Rhinovirus		Entero?		Kobu		Hepato		Parecho	
	SVV	EMCV	TMEV	FMDV-O	ERAV	ERBV	PTV-1	PV-1	CV-A21	CV-A16	CV-B5	EV-70	SEV-A	BEV-1	PEV-9	HRV-14	HRV-2	SV2	PEV-8	AIV	BKV	HAV	AEV	HPeV-1	LV
SVV	100	58	58	47	48	42	40	33	32	30	30	32	31	31	32	34	31	32	31	33	32	24	24	23	23
EMCV	58	100	65	43	46	46	38	31	30	29	30	29	31	30	31	32	32	31	31	35	33	24	25	21	24
TMEV	58	65	100	42	47	42	38	30	29	28	30	30	30	30	29	31	31	30	32	34	32	24	26	22	25
FMDV-O	47	43	42	100	50	37	39	31	31	30	30	31	32	28	31	32	31	31	31	35	35	22	24	21	23
ERAV	48	46	47	50	100	41	37	29	29	29	29	31	31	29	29	31	30	29	30	34	35	25	24	22	24
ERBV	42	46	42	37	41	100	40	33	33	33	34	34	34	33	35	35	35	34	35	33	34	21	25	23	26
PTV-1	40	38	38	39	37	40	100	34	34	32	34	34	35	31	35	34	34	36	35	31	31	27	23	22	23
PV-1	33	31	30	31	29	33	34	100	98	69	75	76	67	68	71	64	57	56	55	33	32	27	27	23	27
CV-A21	32	30	29	31	29	33	34	98	100	68	75	75	66	68	71	64	57	56	55	33	32	27	26	23	27
CV-A16	30	29	28	30	29	33	32	69	68	100	67	65	59	62	66	61	54	55	53	33	35	27	26	23	26
CV-B5	30	30	30	30	29	34	34	75	75	67	100	76	69	70	71	66	57	57	55	32	32	25	25	23	27
EV-70	32	29	30	31	31	34	34	76	75	65	76	100	65	67	68	63	58	58	55	33	35	25	26	22	26
SEV-A	31	31	30	32	31	34	35	67	66	59	69	65	100	64	65	58	56	55	53	33	33	25	27	25	26
BEV-1	31	30	30	28	29	33	31	68	68	62	70	67	64	100	75	60	57	54	53	31	31	25	26	25	27
PEV-9	32	31	29	31	29	35	35	71	71	66	71	68	65	75	100	64	58	58	54	32	33	29	27	23	28
HRV-14	34	32	31	32	31	35	34	64	64	61	66	63	58	60	64	100	56	53	54	34	34	27	28	24	27
HRV-2	31	32	31	31	30	35	34	57	57	54	57	58	56	57	58	56	100	53	54	31	32	25	27	23	27
SV2	32	31	30	31	29	34	36	56	56	55	57	58	55	54	58	53	53	100	67	35	36	27	28	21	26
PEV-8	31	31	32	31	30	35	35	55	55	53	55	55	53	53	54	54	54	67	100	33	33	26	28	26	28
AIV	33	35	34	35	34	33	31	33	33	33	32	33	33	31	32	34	31	35	33	100	75	25	27	23	23
BKV	32	33	32	35	35	34	31	32	32	35	32	35	33	31	33	34	32	36	33	75	100	25	27	23	24
HAV	24	24	24	22	25	21	27	27	27	27	25	25	25	25	29	27	25	27	26	25	25	100	40	24	23
AEV	24	25	26	24	24	25	23	27	26	26	25	26	27	26	27	28	27	28	28	27	27	40	100	26	24
HPeV-1	23	21	22	21	22	23	22	23	23	23	23	22	25	25	23	24	23	21	26	23	23	24	26	100	50
LV	23	24	25	23	24	26	23	27	27	26	27	26	26	27	28	27	27	26	28	23	24	23	24	50	100

Fig. 44 (49/99)

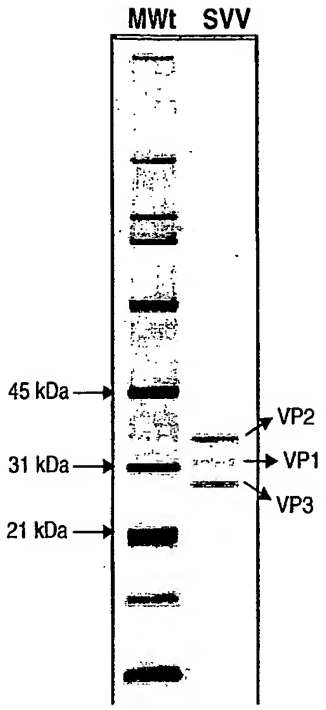


Fig. 45 (50/99)

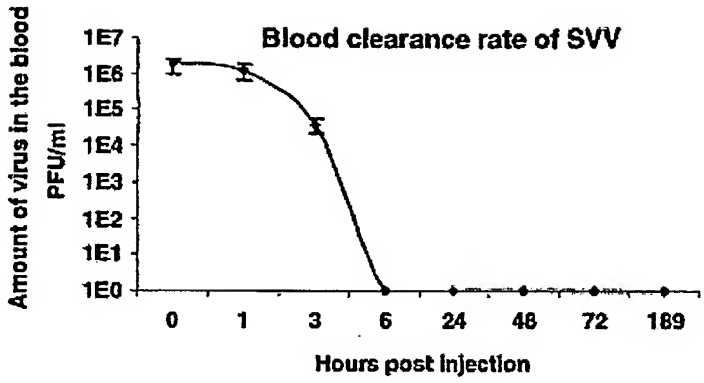


FIG. 46A

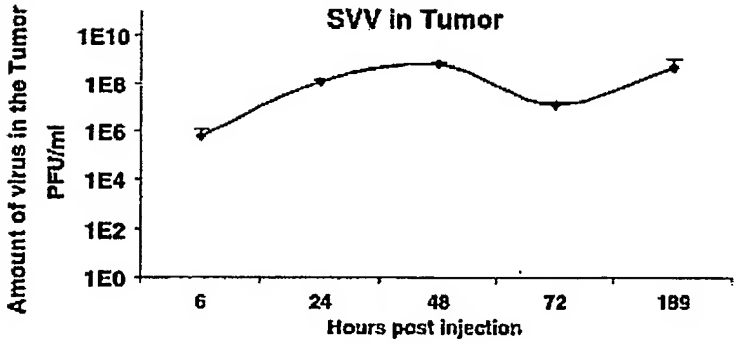


FIG. 46B

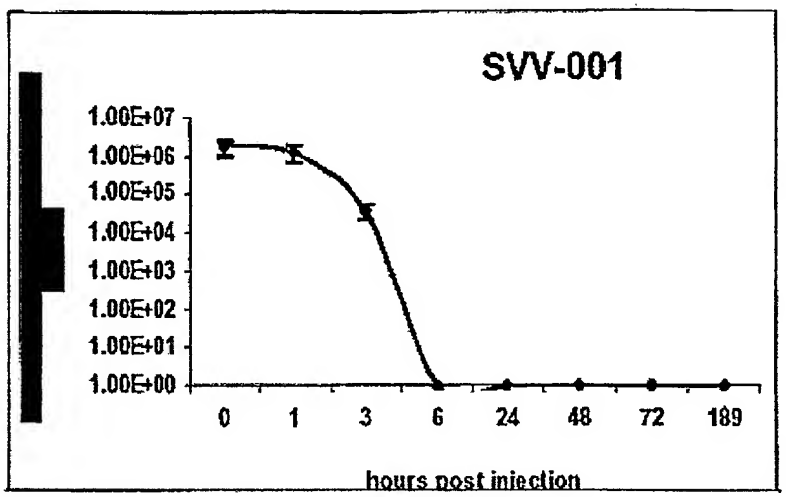


Fig. 46C

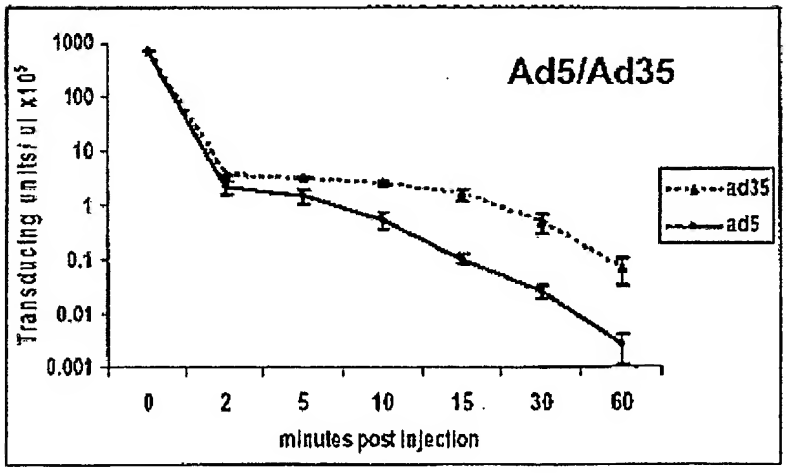


Fig. 46D

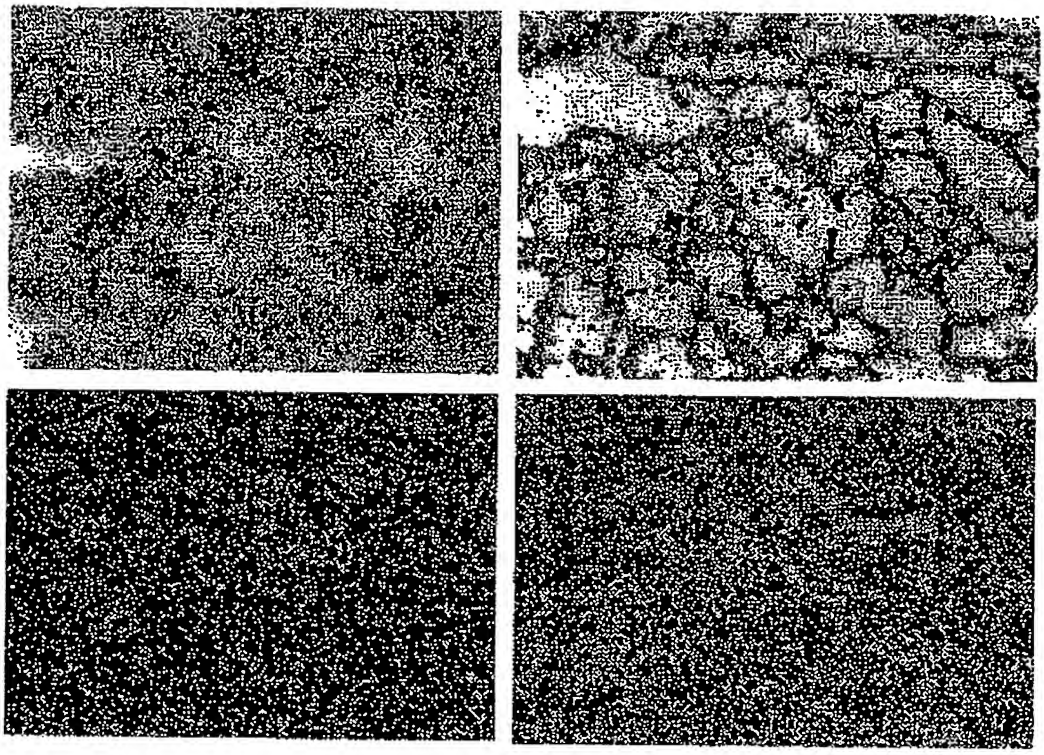


Fig. 47 (53/99)

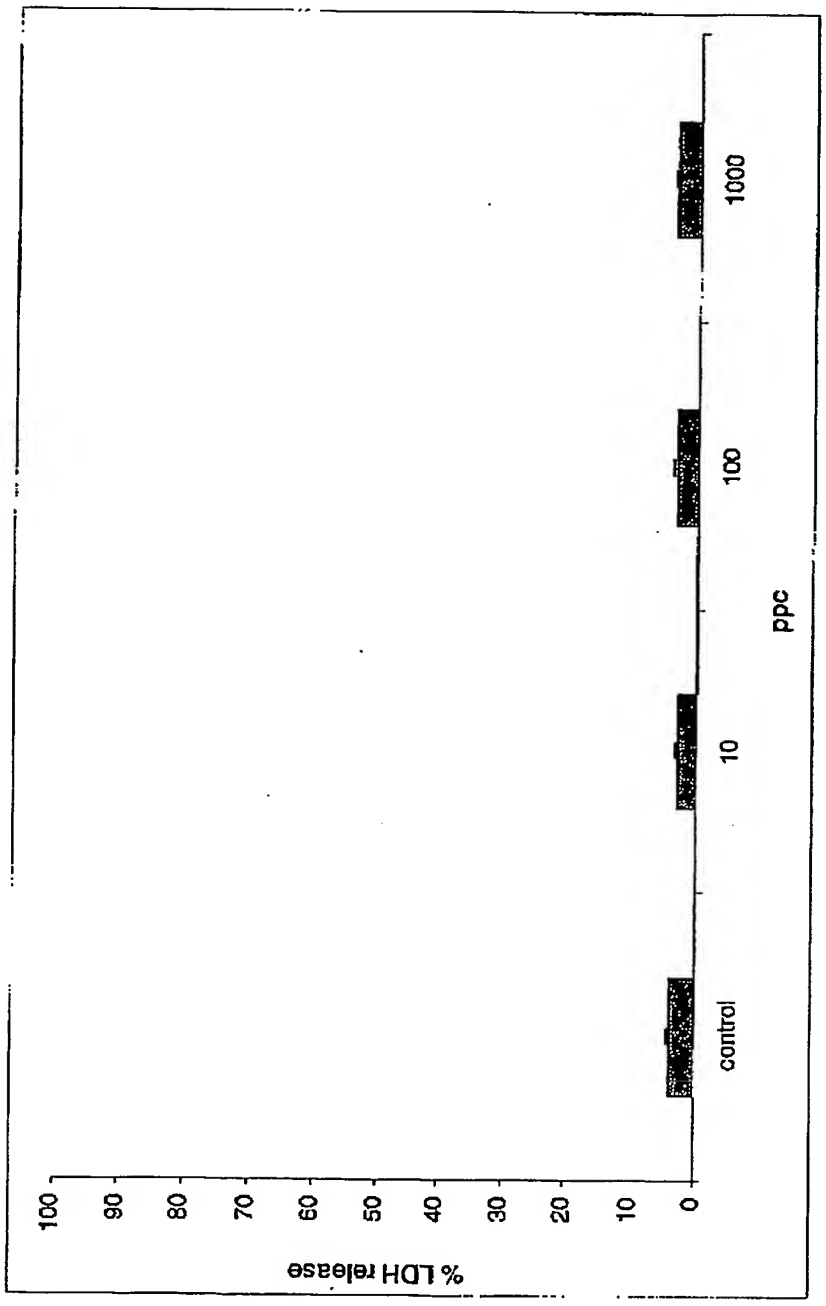

% LDH release
100
90
80
70
60
50
40
30
20
10
0
control
10
100
1000
ppc

Fig. 48

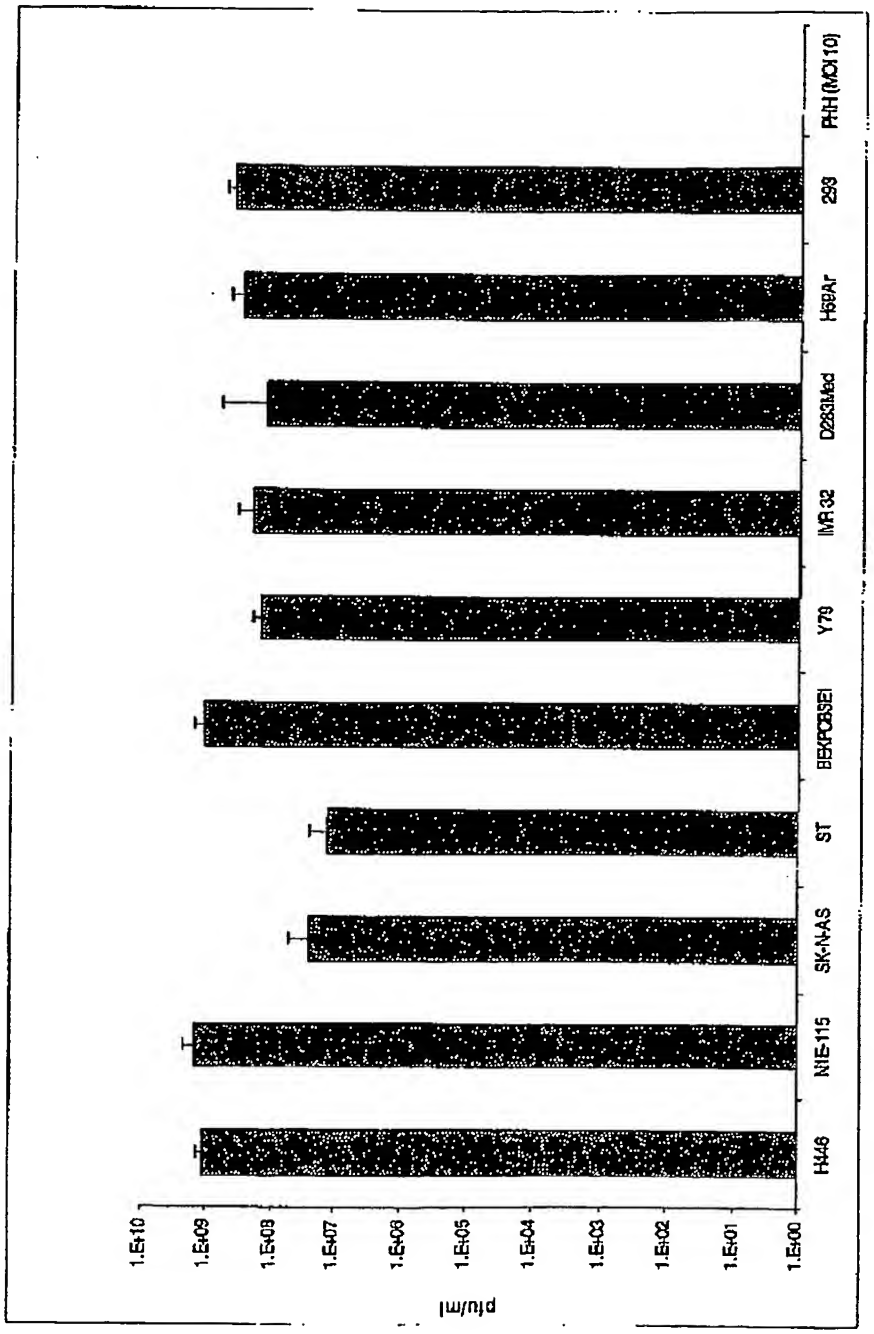

pfu/ml
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
H446
NIE-115
SK-N-AS
ST
BE(P)C8SEI
Y79
IMR 32
D283Med
H69Ar
293
PHH (MOI 10)

Fig. 49

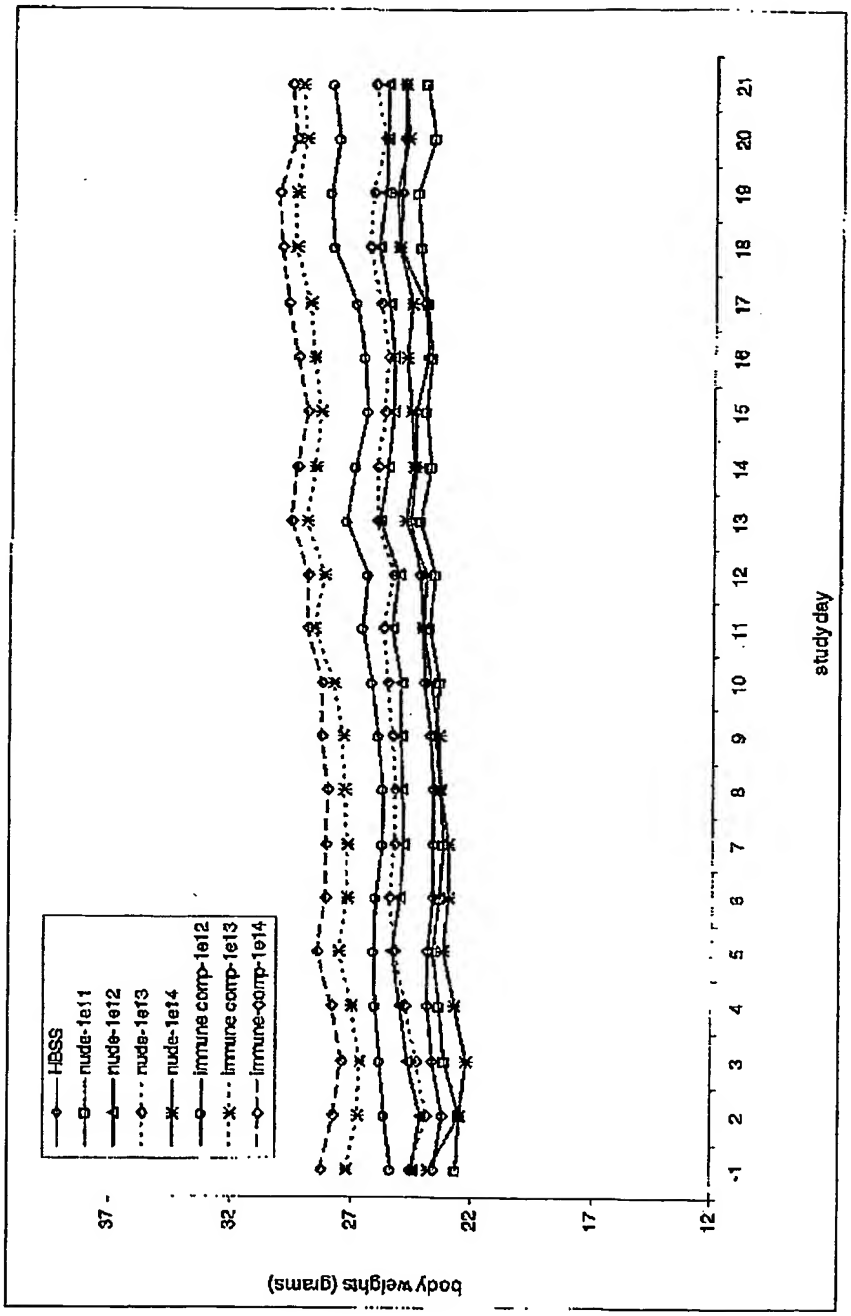

HBSS
nude-1e11
nude-1e12
nude-1e13
nude-1e14
immune comp-1e12
immune comp-1e13
immune comp-1e14
body weights (grams)
37
32
27
22
17
12
-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
study day

Fig. 50

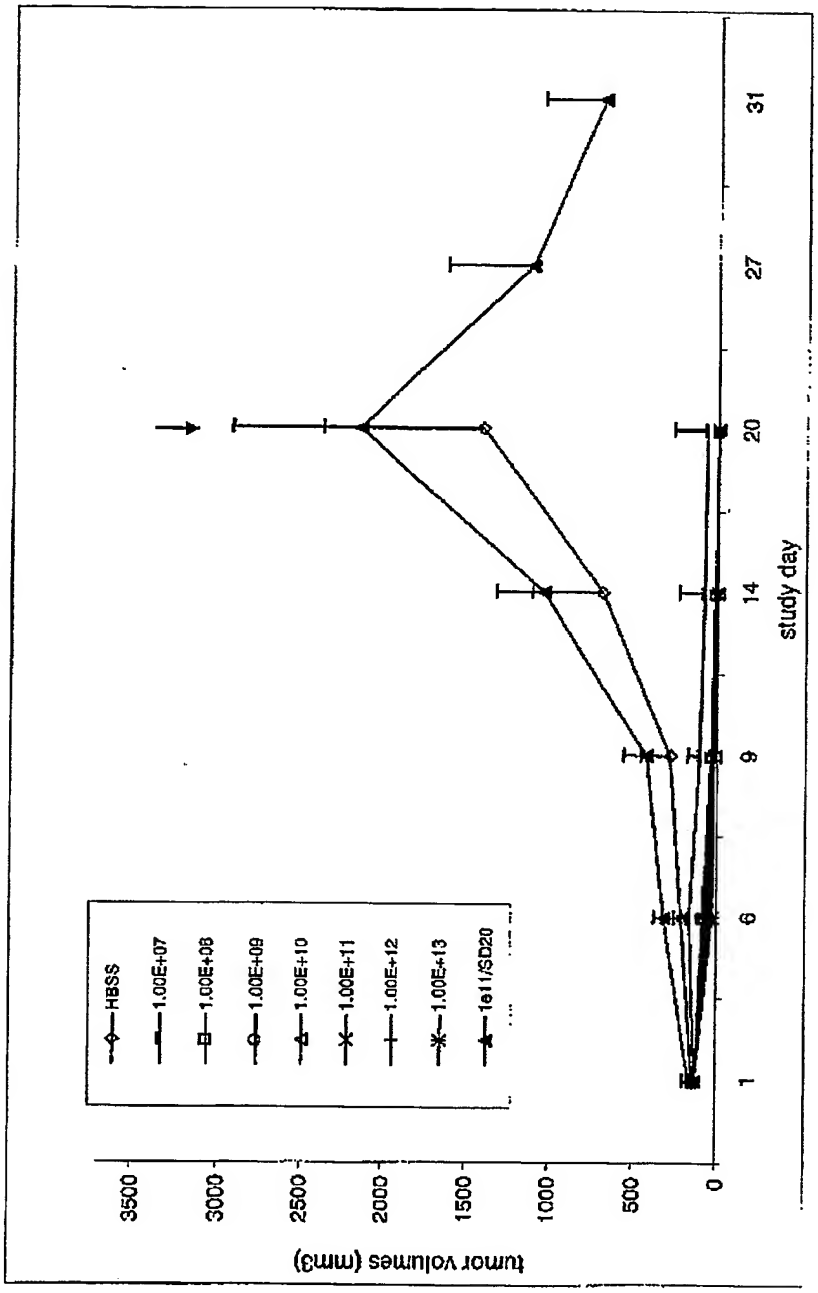

tumor volumes (mm3)
3500
3000
2500
2000
1500
1000
500
0
1
6
9
14
20
27
31
study day
HBSS
1.00E+07
1.00E+08
1.00E+09
1.00E+10
1.00E+11
1.00E+12
1.00E+13
1e11/SD20

Fig. 51

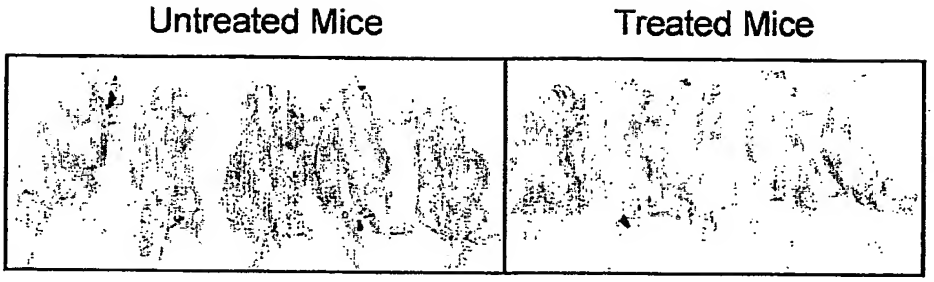


Fig. 52

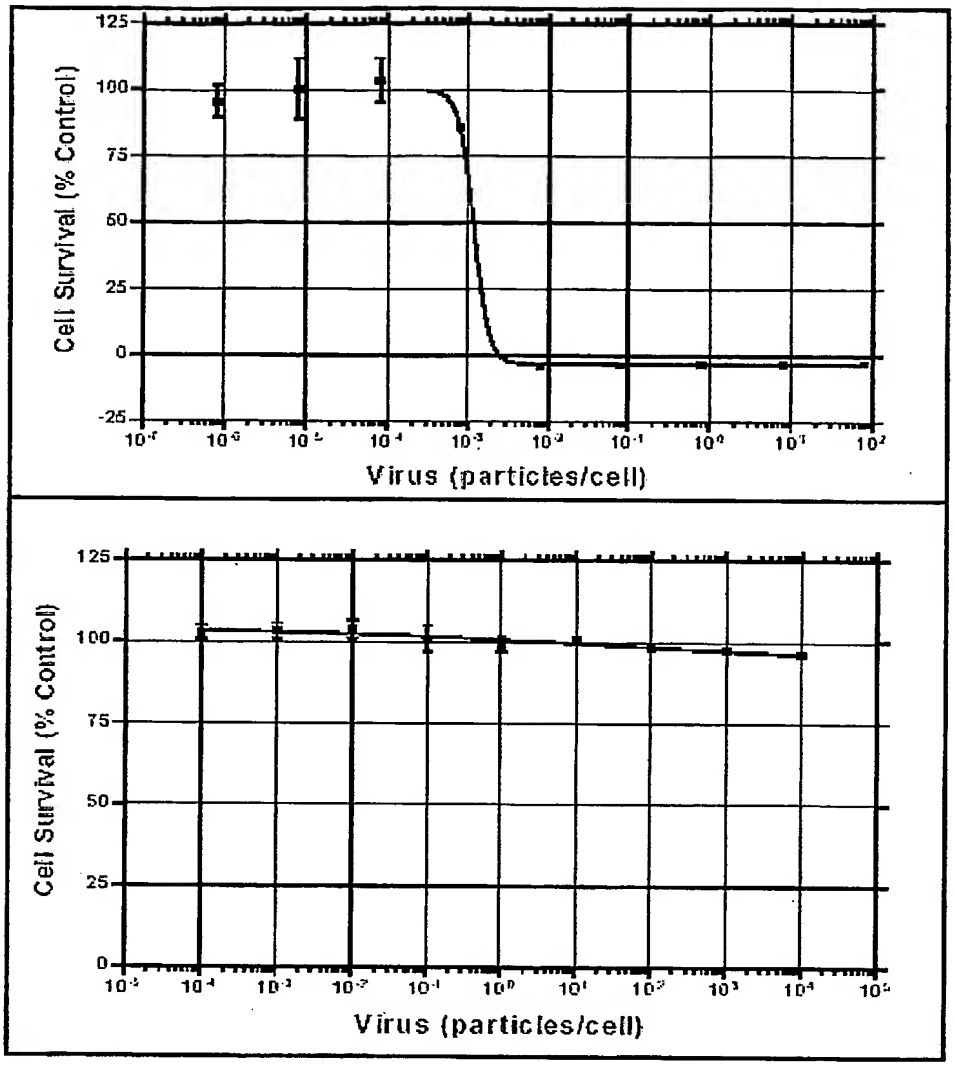
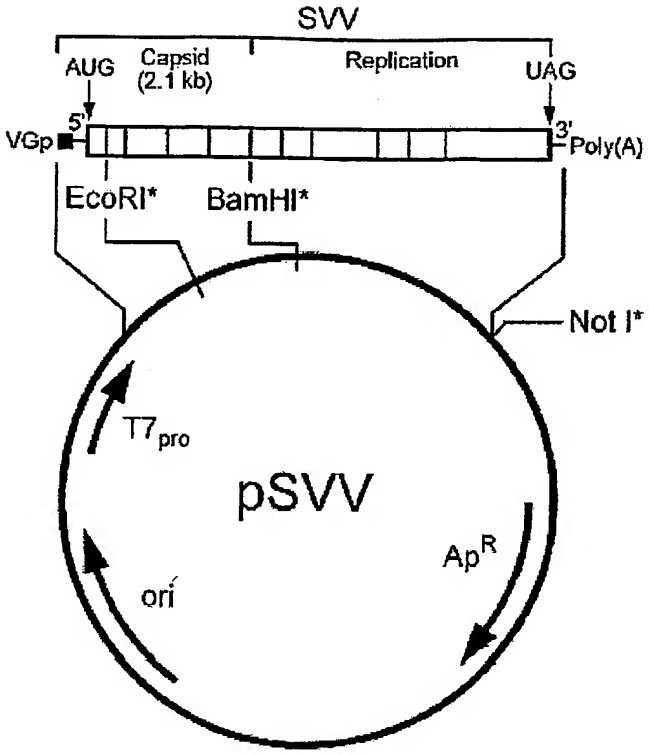

Cell Survival (% Control)
125
100
75
50
25
0
-25
Virus (particles/cell)
Cell Survival (% Control)
125
100
75
50
25
0
Virus (particles/cell)

Fig. 53



*Unique cutters to be defined

Fig. 54

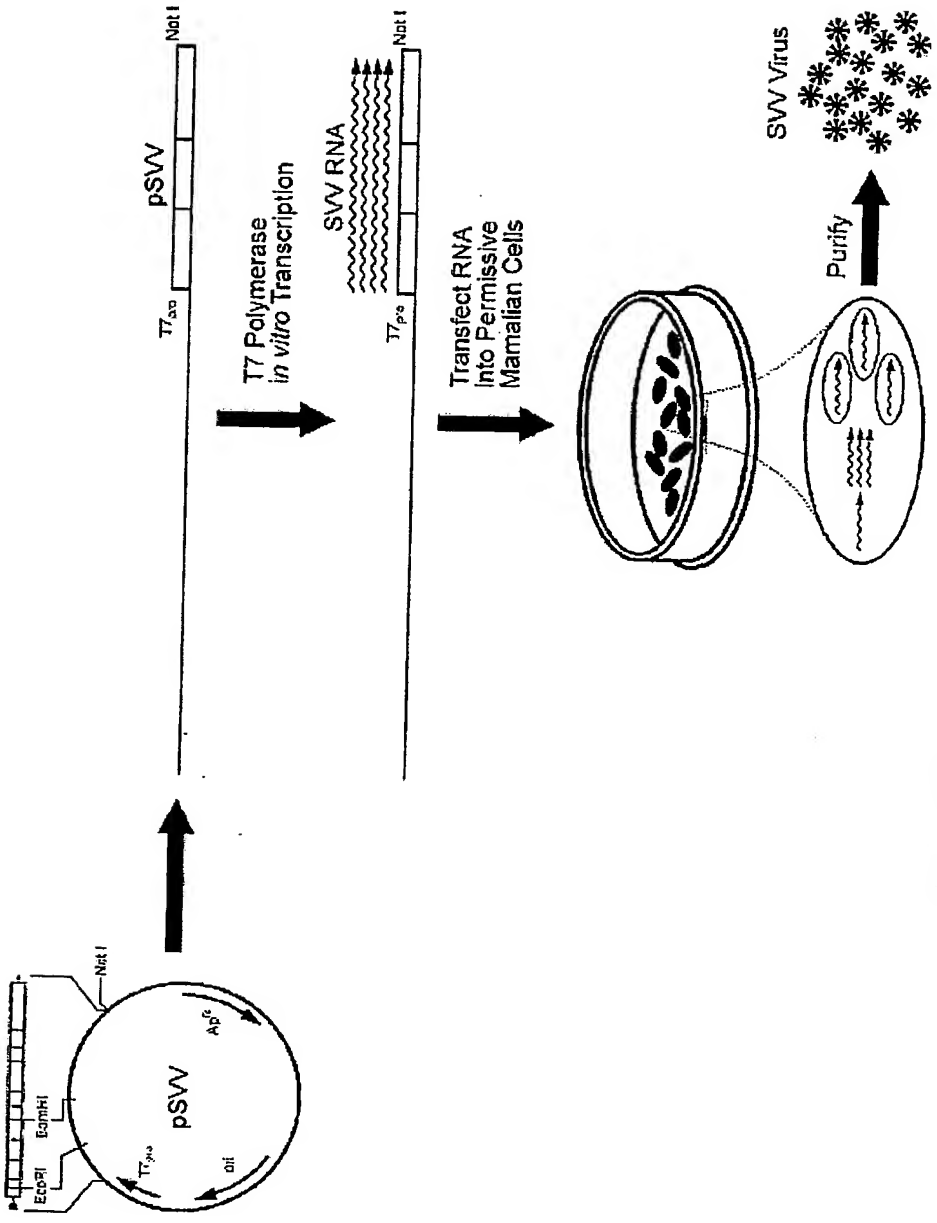

Not I
pSVV
T7 Polymerase
in vitro Transcription
SVV RNA
Not I
Transfect RNA
Into Permissive
Mamalian Cells
SVV Virus
Purify
Not I
BamHI
EcoRI
pSVV
ori

Fig. 55

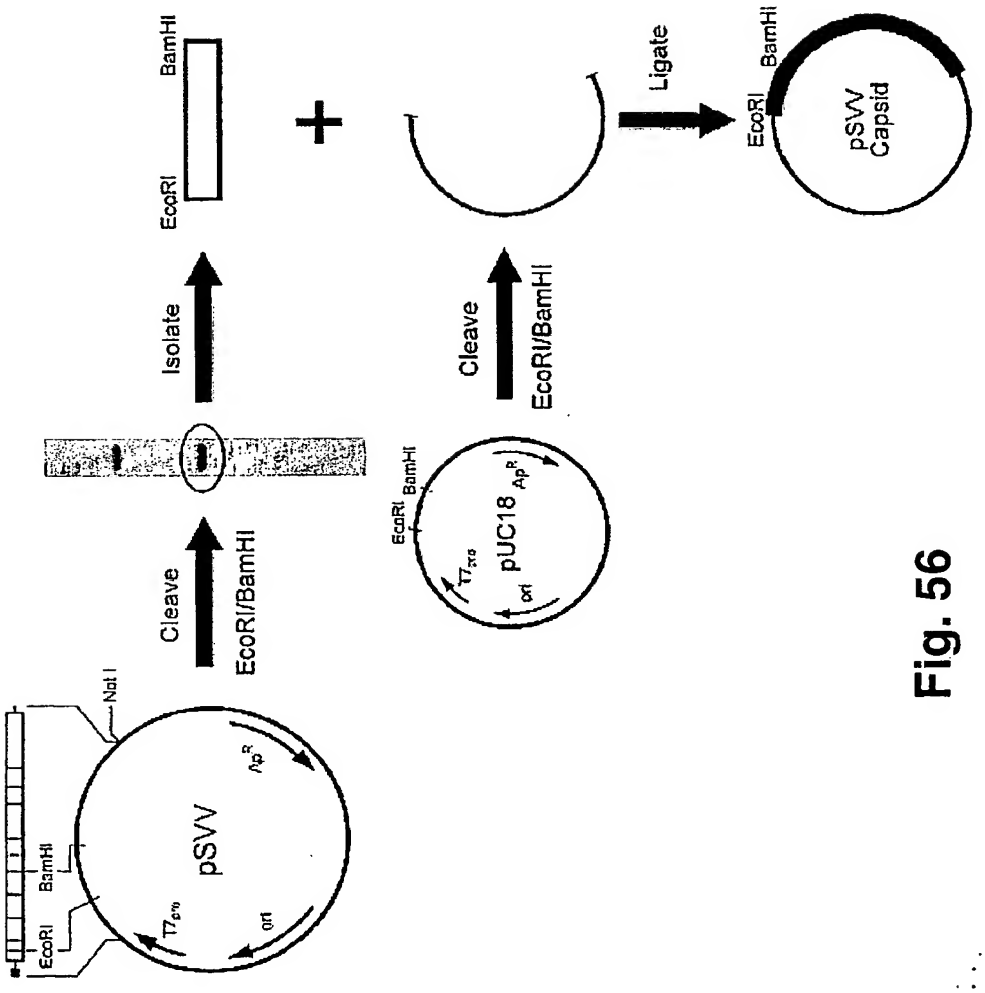

Not I
BamHI
EcoRI
pSVV
Ap^R
ori
Cleave
EcoRI/BamHI
Isolate
BamHI
EcoRI
+
pUC18
Ap^R
ori
Cleave
EcoRI/BamHI
Ligate
BamHI
EcoRI
pSVV
Capsid

Fig. 56

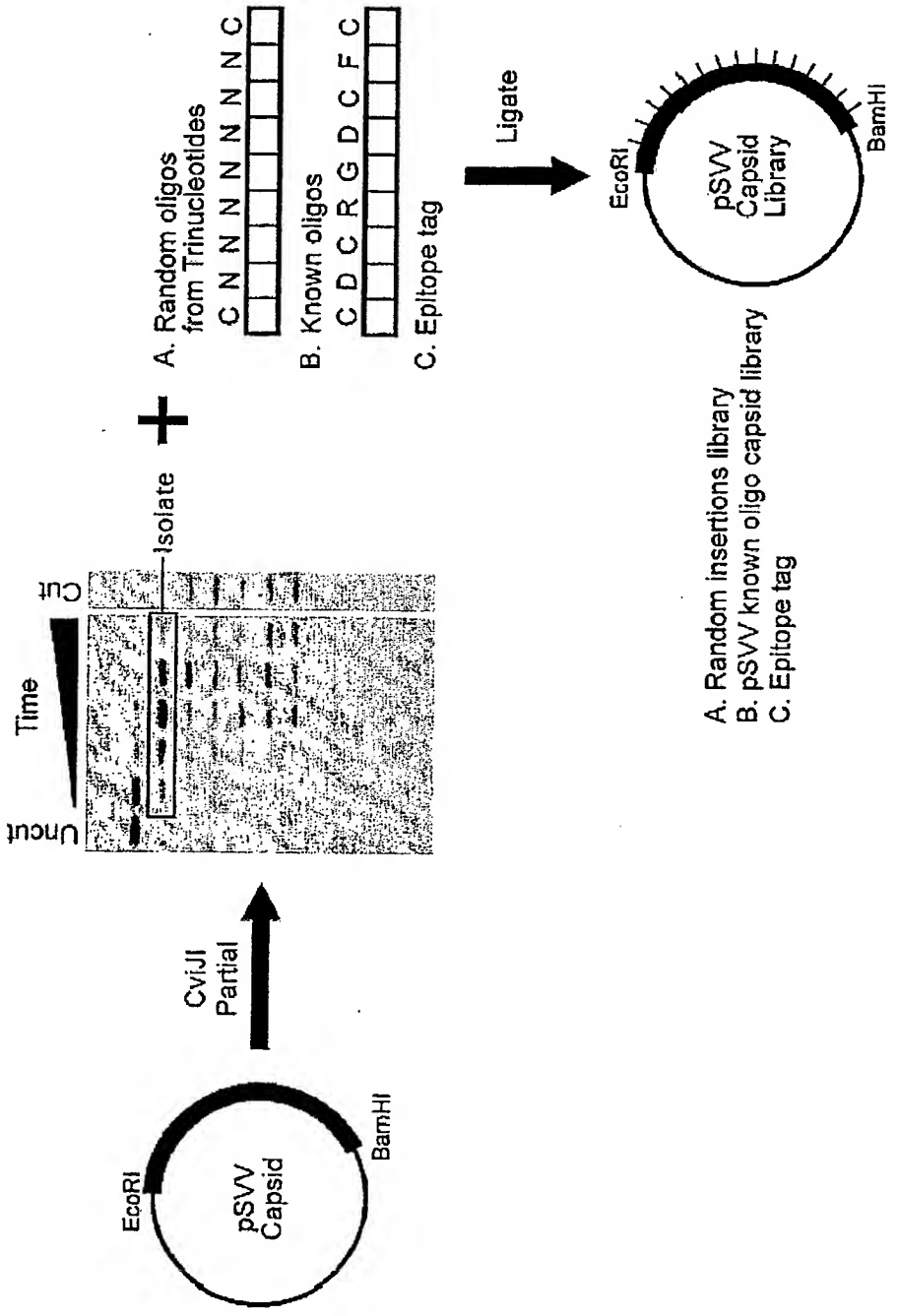

EcoRI
pSVV Capsid
BamHI
CviJI Partial
Uncut
Time
Cut
Isolate
A. Random oligos from Trinucleotides
C N N N N N N N N C
B. Known oligos
C D C R G D C F C
C. Epitope tag
Ligate
EcoRI
pSVV Capsid Library
BamHI
A. Random insertions library
B. pSVV known oligo capsid library
C. Epitope tag

Fig. 57

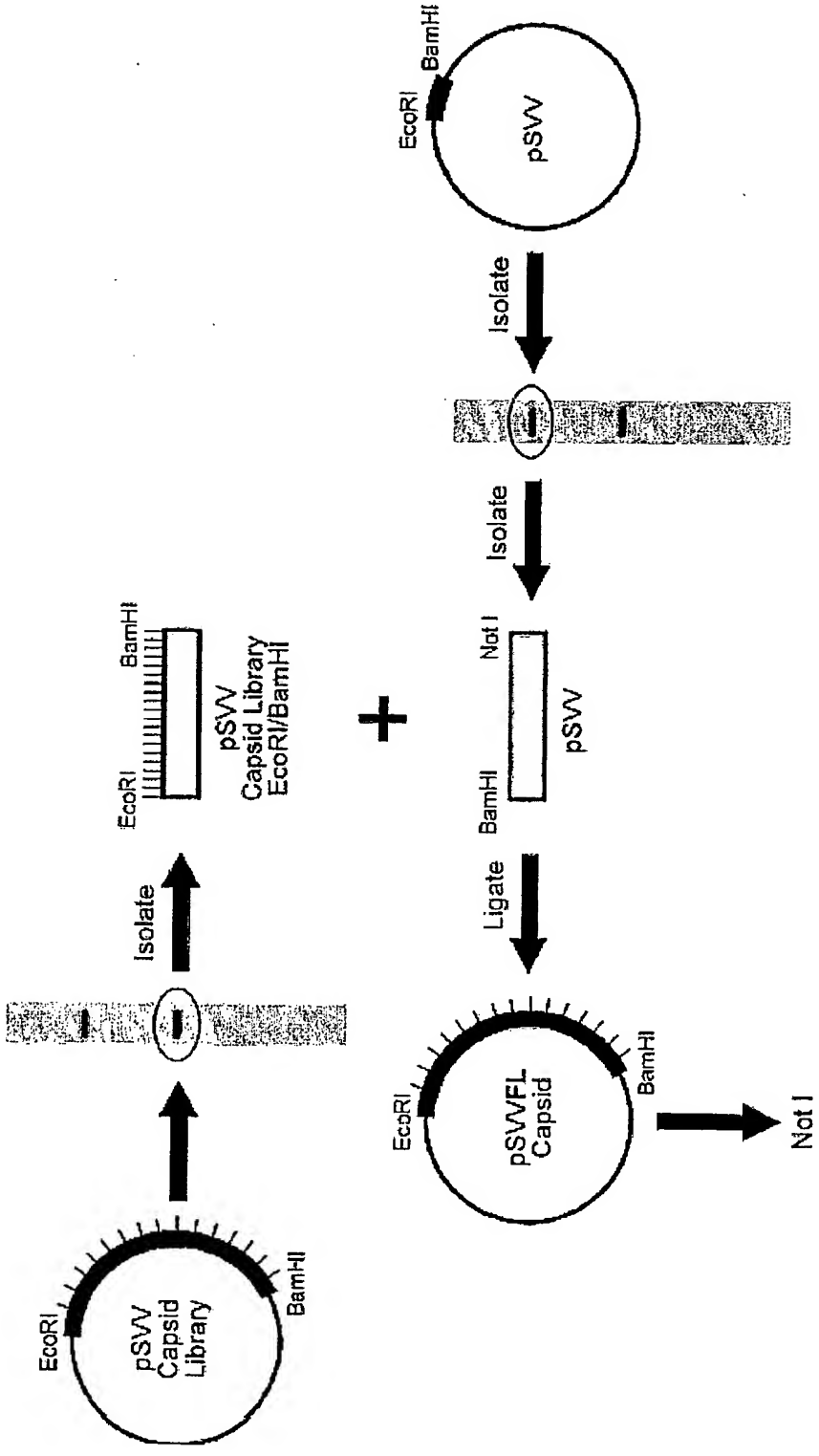

EcoRI
BamHI
pSVV
Isolate
Isolate
Not I
BamHI
pSVV
BamHI
EcoRI
pSVV
Capsid Library
EcoRI/BamHI
+
Isolate
Ligate
EcoRI
BamHI
pSVVFL
Capsid
Not I
EcoRI
BamHI
pSVV
Capsid
Library

Fig. 58

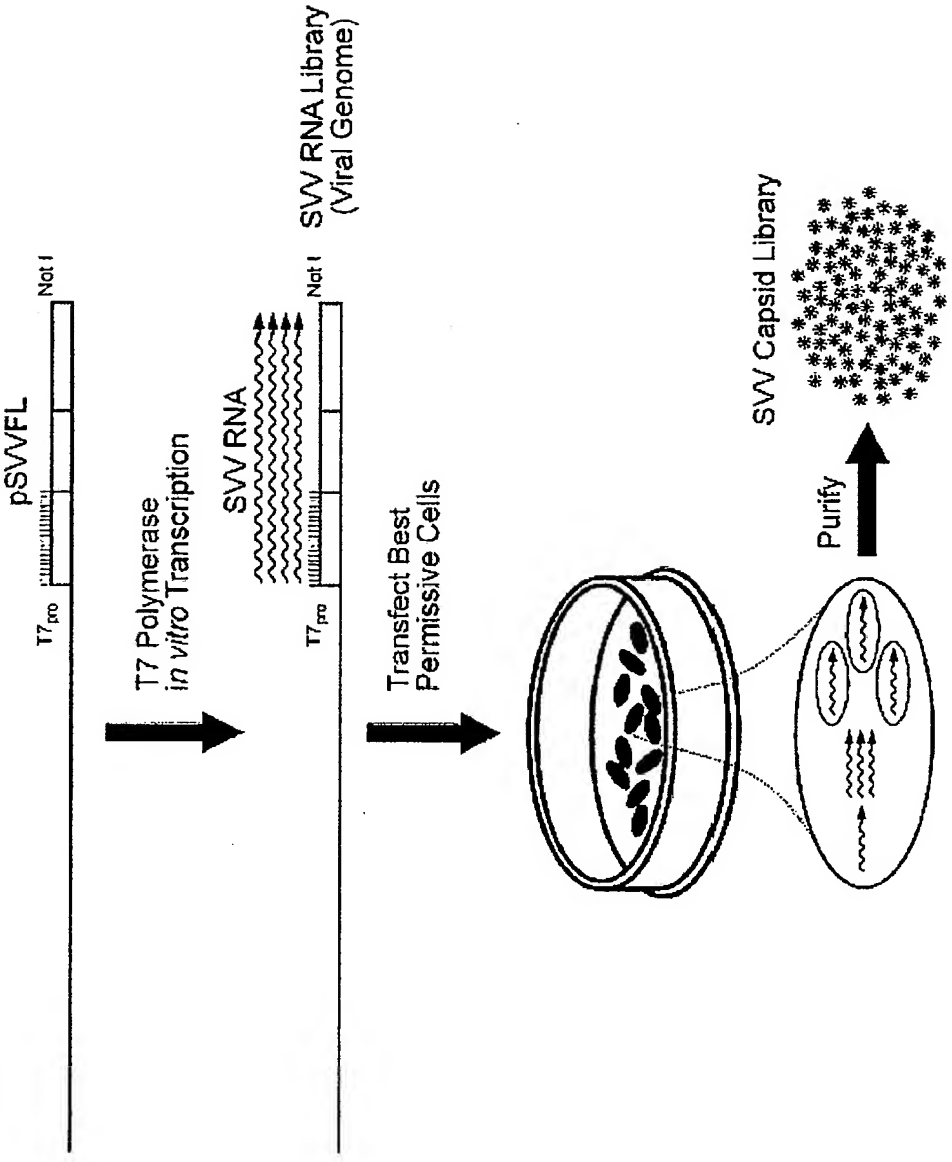

pSVVFL
Not I
T7pro
T7 Polymerase
in vitro Transcription
SVV RNA
SVV RNA Library
(Viral Genome)
Not I
T7pro
Transfect Best
Permissive Cells
SVV Capsid Library
Purify

Fig. 59

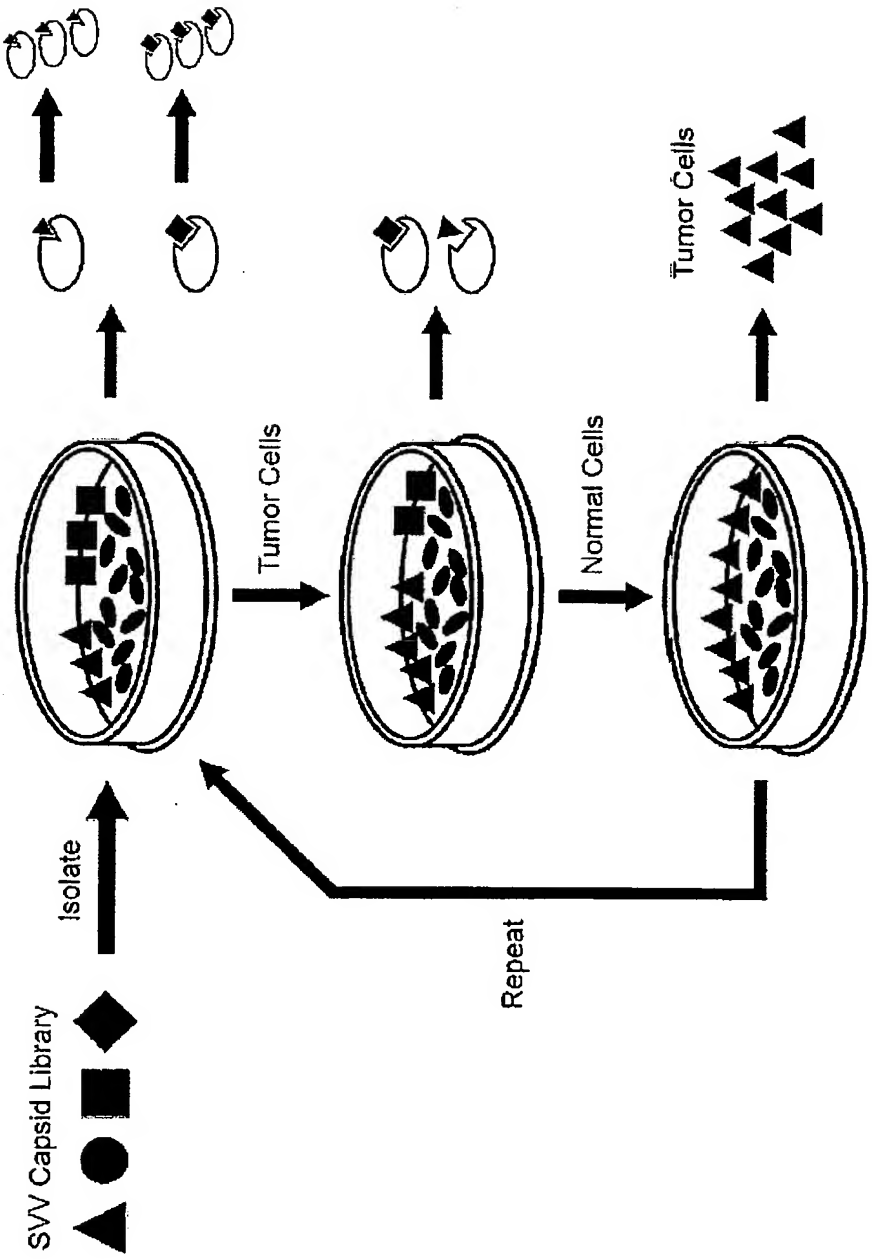

SVV Capsid Library
Isolate
Tumor Cells
Normal Cells
Tumor Cells
Repeat

Fig. 60

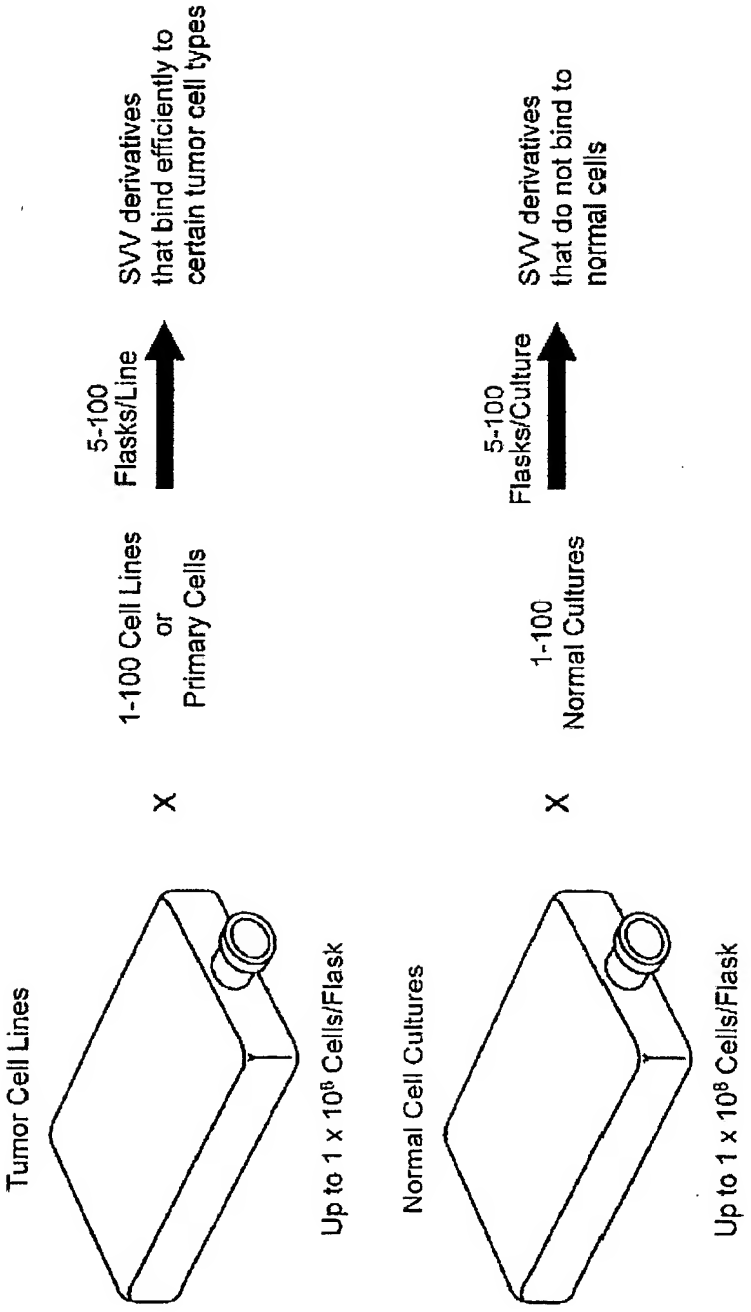

Tumor Cell Lines
Up to 1 x 10⁸ Cells/Flask
X
1-100 Cell Lines or Primary Cells
5-100 Flasks/Line
SVV derivatives that bind efficiently to certain tumor cell types
Normal Cell Cultures
Up to 1 x 10⁸ Cells/Flask
X
1-100 Normal Cultures
5-100 Flasks/Culture
SVV derivatives that do not bind to normal cells

Fig. 61

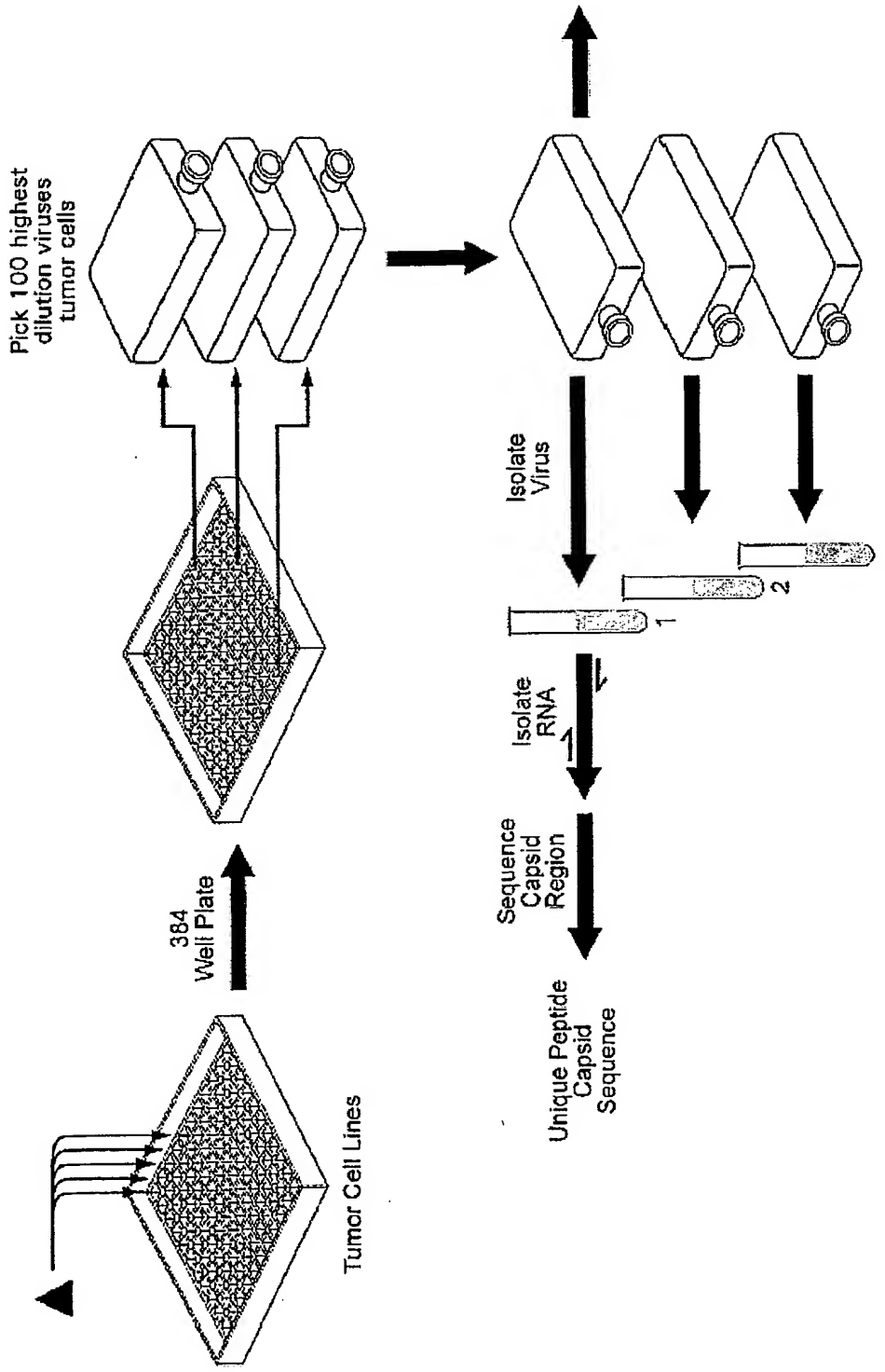

Pick 100 highest dilution viruses tumor cells
384 Well Plate
Tumor Cell Lines
Isolate Virus
1
2
Isolate RNA
Sequence Capsid Region
Unique Peptide Capsid Sequence

Fig. 62

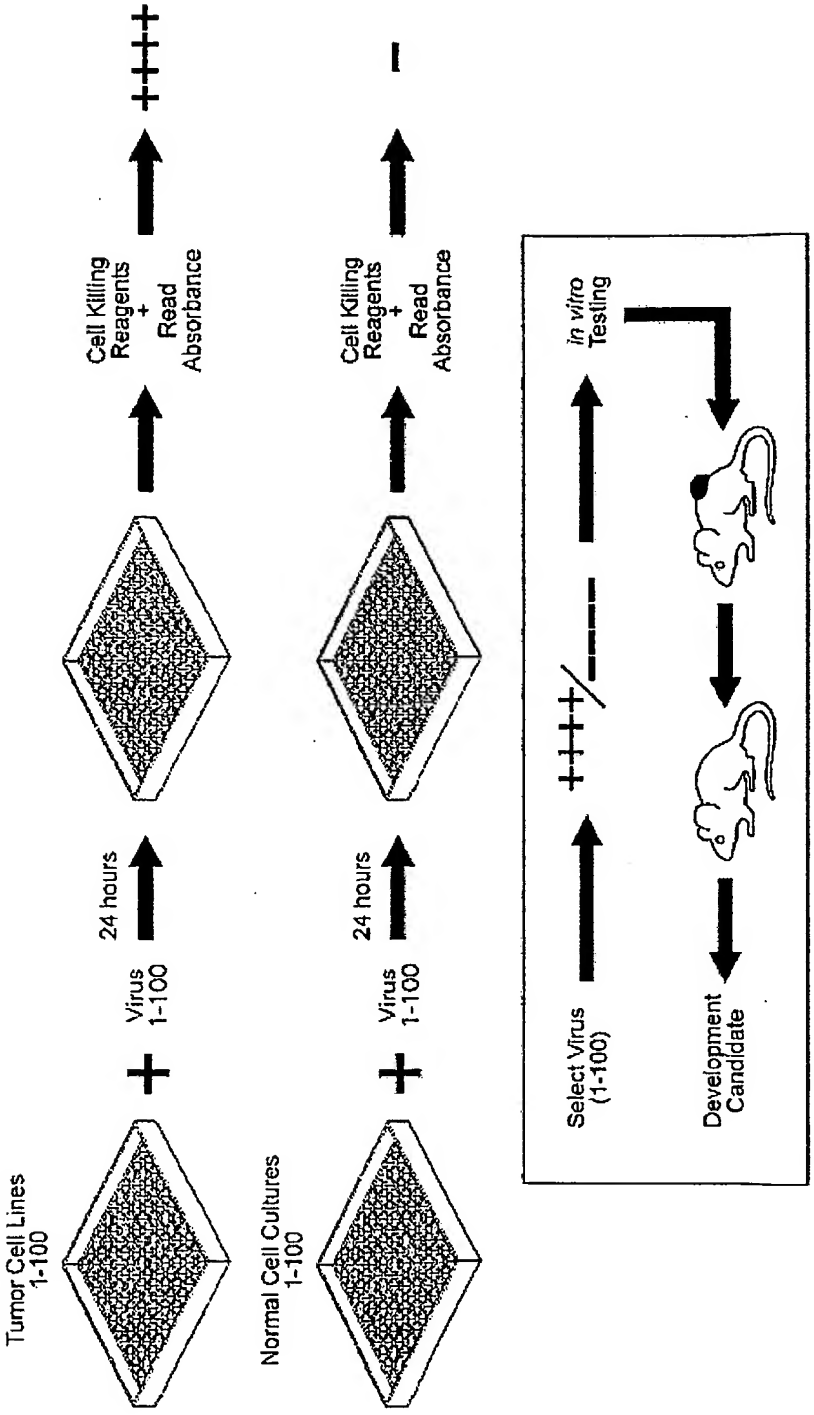

Tumor Cell Lines
1-100
Normal Cell Cultures
1-100
Virus
1-100
24 hours
Cell Killing
Reagents
+
Read
Absorbance
++++
-
Select Virus
(1-100)
++++/---
in vitro
Testing
Development
Candidate

Fig. 63

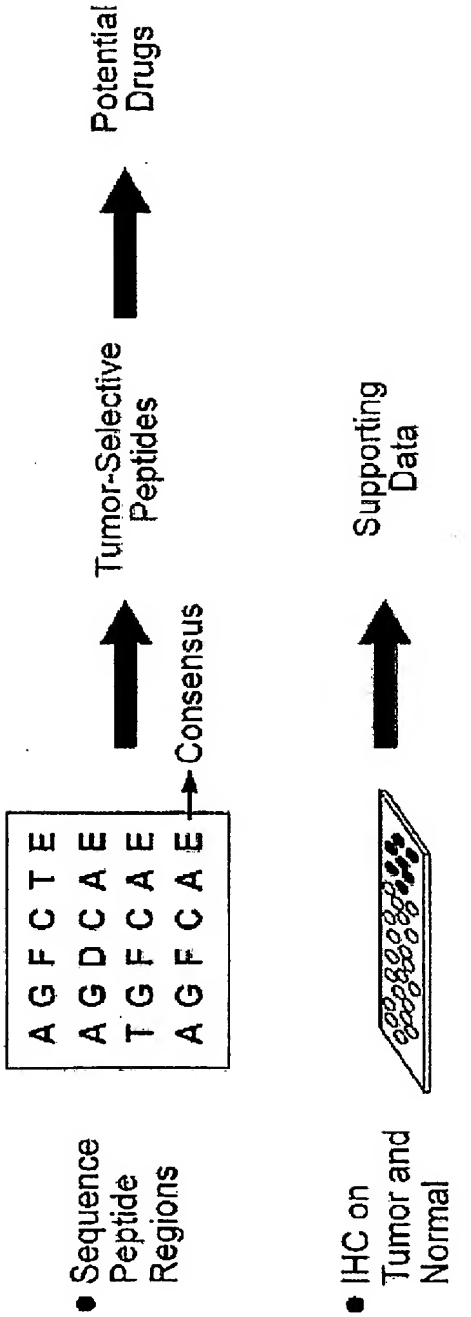

Potential Drugs
Tumor-Selective Peptides
Consensus
A G F C T E
A G D C A E
T G F C A E
A G F C A E
Supporting Data
• Sequence Peptide Regions
• IHC on Tumor and Normal

Fig. 64

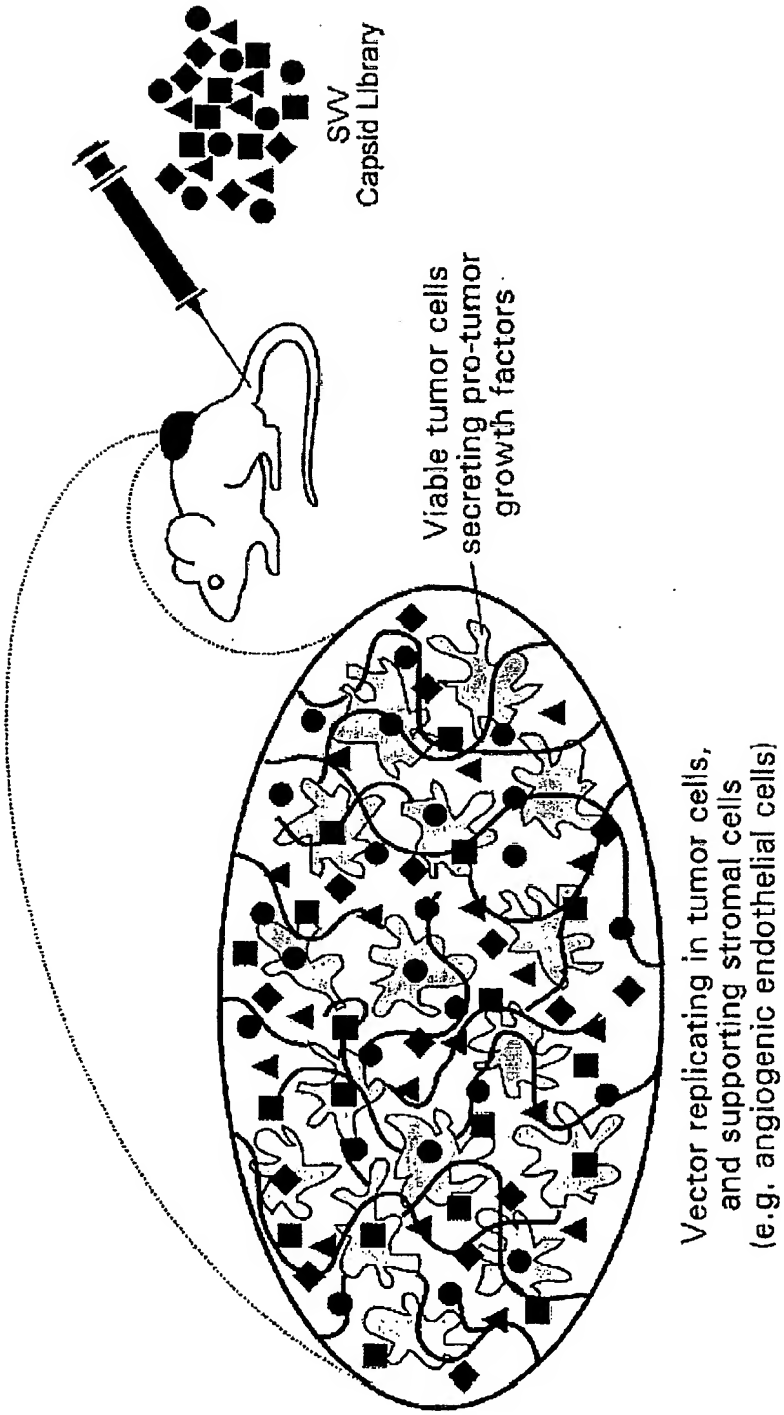

SVV
Capsid Library
Viable tumor cells
secreting pro-tumor
growth factors
Vector replicating in tumor cells,
and supporting stromal cells
(e.g. angiogenic endothelial cells)

Fig. 65

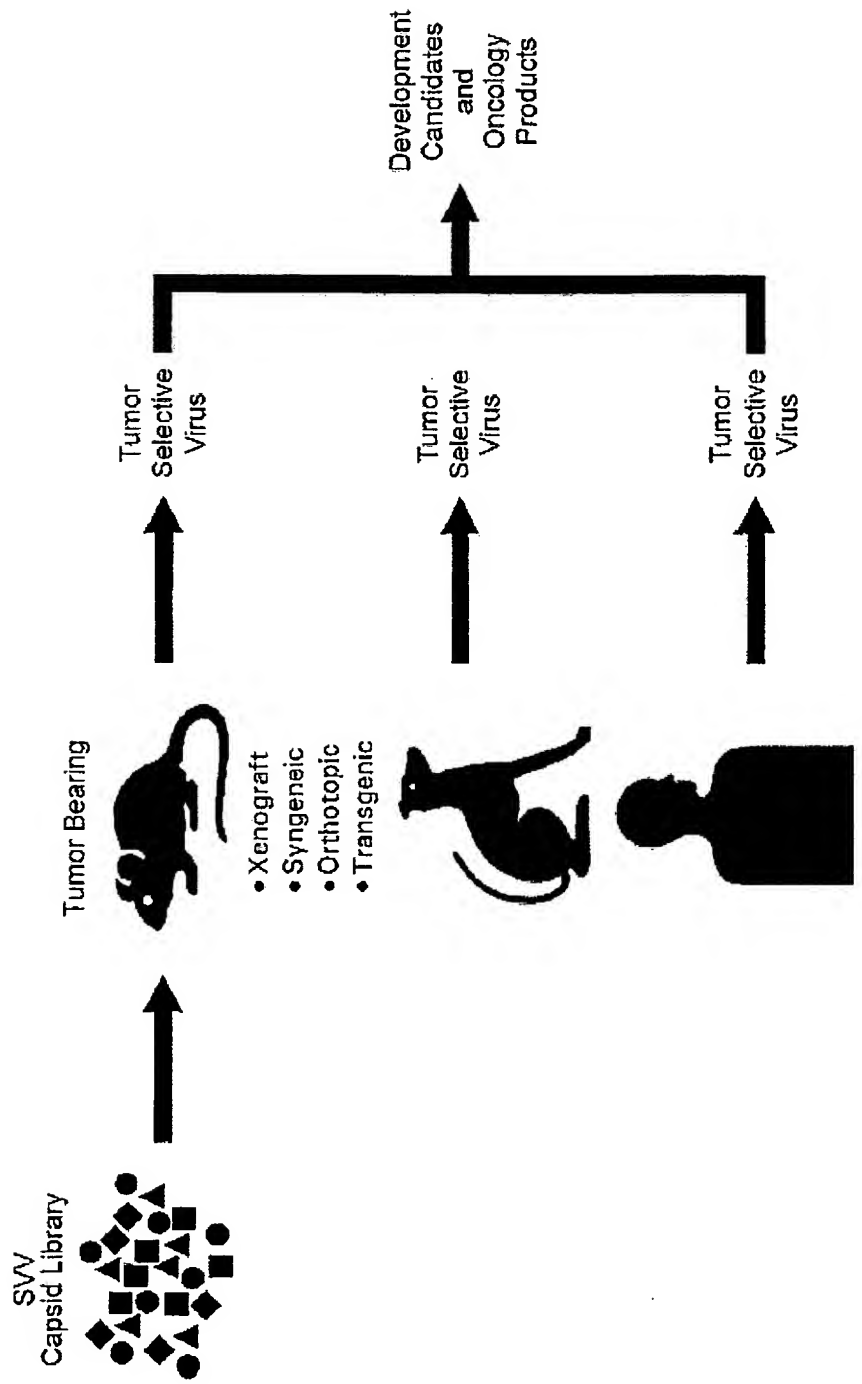

SVV
Capsid Library
Tumor Bearing
• Xenograft
• Syngeneic
• Orthotopic
• Transgenic
Tumor
Selective
Virus
Tumor
Selective
Virus
Tumor
Selective
Virus
Development
Candidates
and
Oncology
Products

Fig. 66

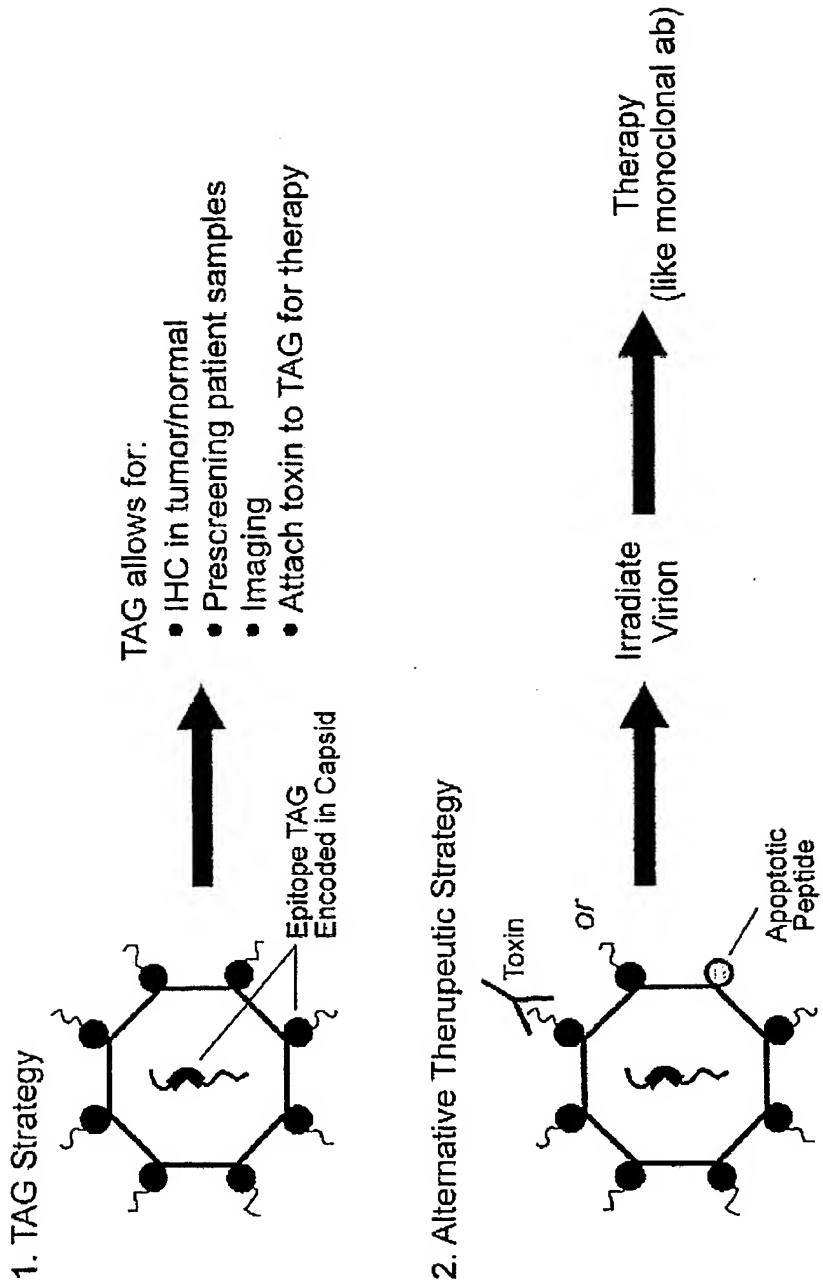

1. TAG Strategy
Epitope TAG
Encoded in Capsid
TAG allows for:
• IHC in tumor/normal
• Prescreening patient samples
• Imaging
• Attach toxin to TAG for therapy
2. Alternative Therupeutic Strategy
Toxin
or
Apoptotic
Peptide
Irradiate
Virion
Therapy
(like monoclonal ab)

Fig. 67

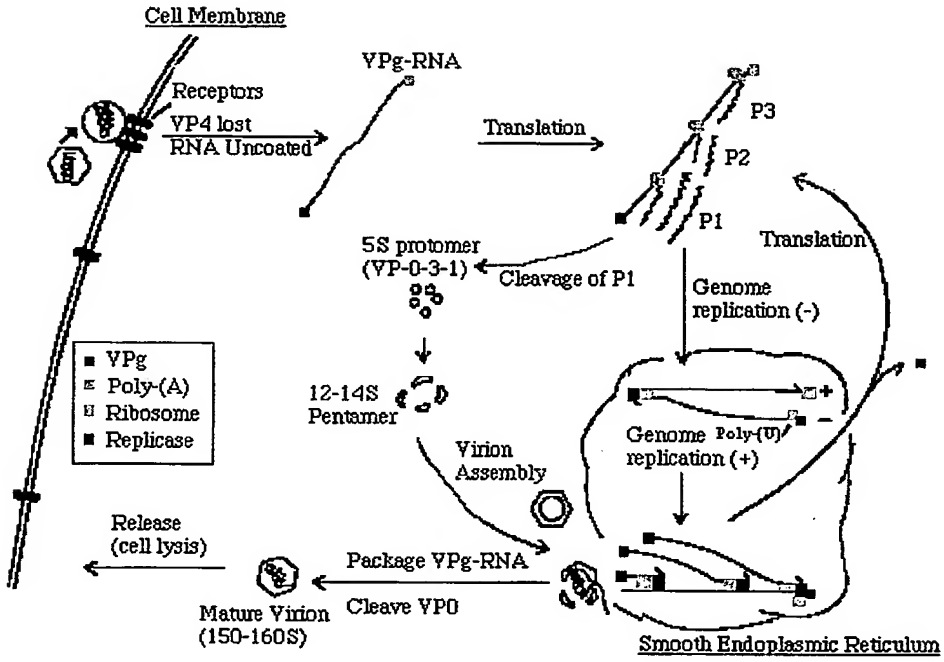

Cell Membrane
Receptors
VP4 lost
RNA Uncoated
VPg-RNA
Translation
P3
P2
P1
Translation
5S protomer
(VP-0-3-1)
Cleavage of P1
Genome
replication (-)
VPg
Poly-(A)
Ribosome
Replicase
12-14S
Pentamer
Genome Poly-(U)
replication (+)
Virion
Assembly
Release
(cell lysis)
Package VPg-RNA
Cleave VP0
Mature Virion
(150-160S)
Smooth Endoplasmic Reticulum

Fig. 68

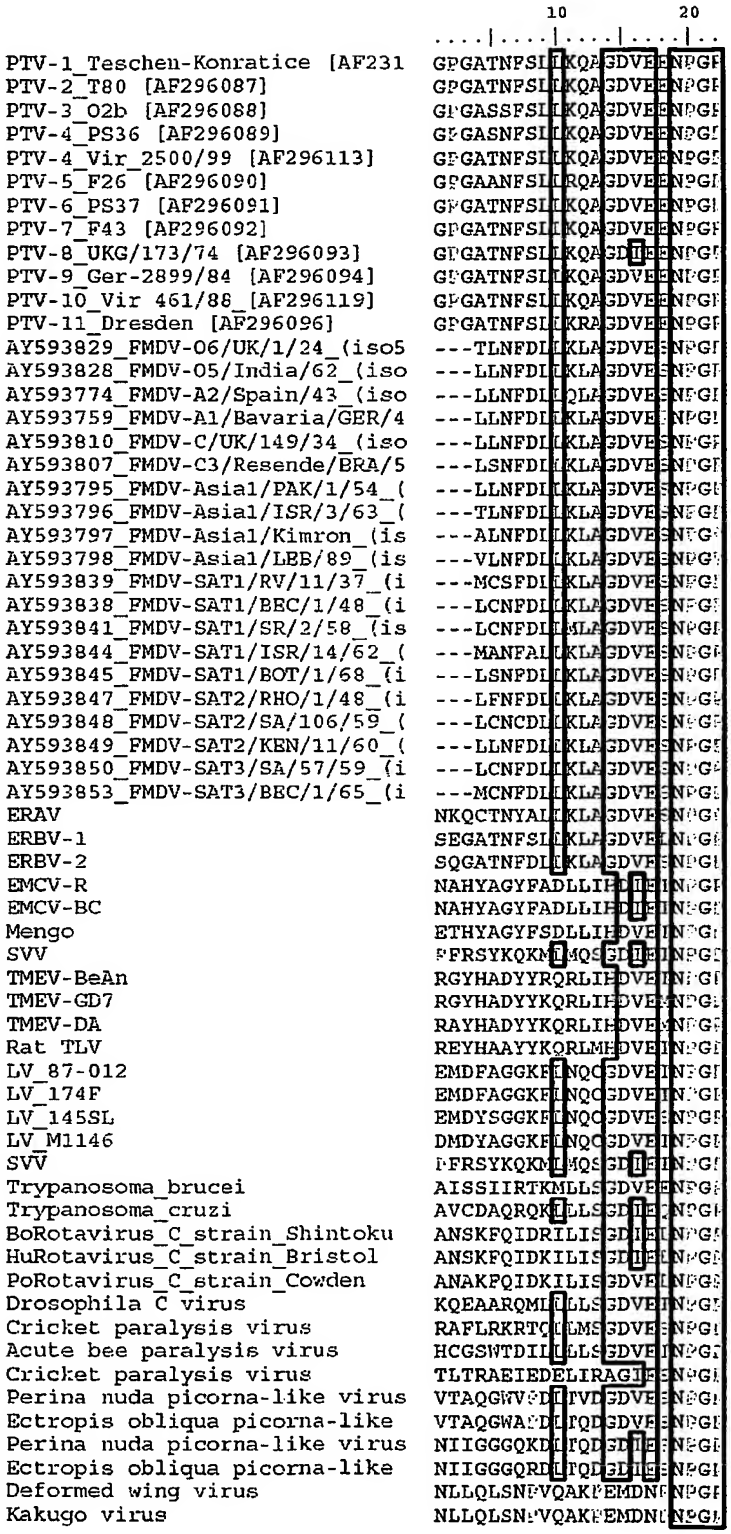
Polypeptide lengths of Seneca Valley virus compared to other picornaviruses

	SVV	EMCV	TMEV	ERBV-1	FMDV-O	ERAV	PTV-1	PV-1	HRV-1B	SV2	PEV-8	DPV	HAV	AEV	AiV	BKV	HPeV-1	LV
Polyprotein	?	2292		2590	2332	2249		2209	2157				2227	2134	2432	2463	2180	2253
P1	?	834		880	736	784		881	857				791	757	846	857	776	820
P2	465	618		616	488	467		575	559				631	590	636	634	598	606
P3	785	773		875	907	789		753	741				805	787	780	785	806	827
Leader	?	67		219	201	209		-	-				-	-	170	187	-	-
VP0	?	326		327	303	310		341	332				245	242	370	367	289	259
VP4	?	70		71	85	80		69	69				23	19	-	-	-	-
VP2	?	256		256	218	230		272	263				222	223	-	-	-	-
VP3	239	231		229	220	226		238	238				246	245	223	223	253	244
VP1	259	277		324	211	248		302	287				300	270	253	267	234	317
2A	14	143		16	18	16		149	142				45	13	136	134	147	135
2B	128	150		283	154	136		97	95				251	251	165	165	122	138
2C	323	325		317	318	315		329	322				335	326	335	335	329	333
3A	90	88		133	153	95		87	77				74	65	95	94	117	130
3B	22	20		21	23-24†	24		22	21				23	21	27	30	20	29
3C	211	205		252	213	205		183	183				219	215	190	192	200	198
3D	462	460		469	470	465		461	460				489	486	468	469	469	470

*, assuming that the 20 aa peptide ending in NPG is the C-terminus of VP1.
†, FMDV has three copies of VPg in tandem.
na, these individual polypeptides do not exist in these viruses.

Fig. 69

WO 2005/030139 PCT/US2004/031504


10
20
PTV-1_Teschen-Konratice [AF231
PTV-2_T80 [AF296087]
PTV-3_O2b [AF296088]
PTV-4_PS36 [AF296089]
PTV-4_Vir_2500/99 [AF296113]
PTV-5_F26 [AF296090]
PTV-6_PS37 [AF296091]
PTV-7_F43 [AF296092]
PTV-8_UKG/173/74 [AF296093]
PTV-9_Ger-2899/84 [AF296094]
PTV-10_Vir 461/88_[AF296119]
PTV-11_Dresden [AF296096]
AY593829_FMDV-O6/UK/1/24_(iso5
AY593828_FMDV-O5/India/62_(iso
AY593774_FMDV-A2/Spain/43_(iso
AY593759_FMDV-A1/Bavaria/GER/4
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AY593795_FMDV-Asia1/PAK/1/54_(
AY593796_FMDV-Asia1/ISR/3/63_(
AY593797_FMDV-Asia1/Kimron_(is
AY593798_FMDV-Asia1/LBB/89_(is
AY593839_FMDV-SAT1/RV/11/37_(i
AY593838_FMDV-SAT1/BEC/1/48_(i
AY593841_FMDV-SAT1/SR/2/58_(is
AY593844_FMDV-SAT1/ISR/14/62_(
AY593845_FMDV-SAT1/BOT/1/68_(i
AY593847_FMDV-SAT2/RHO/1/48_(i
AY593848_FMDV-SAT2/SA/106/59_(
AY593849_FMDV-SAT2/KEN/11/60_(
AY593850_FMDV-SAT3/SA/57/59_(i
AY593853_FMDV-SAT3/BEC/1/65_(i
ERAV
ERBV-1
ERBV-2
EMCV-R
EMCV-BC
Mengo
SVV
TMEV-BeAn
TMEV-GD7
TMEV-DA
Rat TLV
LV_87-012
LV_174F
LV_145SL
LV_M1146
SVV
Trypanosoma_brucei
Trypanosoma_cruzi
BoRotavirus_C_strain_Shintoku
HuRotavirus_C_strain_Bristol
PoRotavirus_C_strain_Cowden
Drosophila C virus
Cricket paralysis virus
Acute bee paralysis virus
Cricket paralysis virus
Perina nuda picorna-like virus
Ectropis obliqua picorna-like
Perina nuda picorna-like virus
Ectropis obliqua picorna-like
Deformed wing virus
Kakugo virus

Fig. 70 (76/99)

```
   1 MATTMEQETC AHSLTFEECP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS EGNEGVIINN FYSNQYQNSI DLSANAAGSD PPRTYGQFSN
 121 LFSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTRTQTNKKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPSNYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNADGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGVTENTNAT ADFVAQPVYL PENQTKVAFF
 661 YNRSSPIGAF TVKSGSLESG FAPFSNGTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGISNQISF VVPYNSPLSV LSAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNKR VFCPRPTVFF PWPTSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHVLIQ FNHRGLEVRL FRHGHFWAET RADVILRSKT KQVSFLSNGN
 961 YPSMDSRAPW NPWKNTYQAV LRAEPCRVTM DIYYKRVRPF RLPLVQKEWP VREENVFGLY
1021 RIFNAHYAGY FADLLIHDIE TNPGPFMFRP RKQVFQTQGA AVSSMAQTLL PNDLASKAMG
1081 SAFTALLDAN EDAQKAMKII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI AAKFKTIFIT PPPRFPTISL FQQQSPLKQV
1201 NDIFSLAKNL DWAVKTVEKV VDWFGTWIVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMAA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV
1381 STTNFLPNMA SLERKGTPFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC
```

Fig. 71A (77/99)

1441 SKTEAGYKVL DVERAFRPTG EAPLPCFQNN CLFLEKAGLQ FRDNRTKEII SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQG PVDEVSFHSV VQQLKARQQA TDEQLEELQE AFAKVQERNS

1561 VFSDWLKISA MLCAATLALS QVVKMAKAVK QMVKPDLVRV QLDEQEQGPY NETARVKPKT

1621 LQLLDIQGPN PVMDFEKYVA KHVTAPIGFV YPTGVSTQTC LLVRGRTLVV NRHMAESDWT

1681 SIVVRGVTHA RSTVKILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAG DVLPTGAAPV

1741 TGIMNTDIPM MYTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGSKKI

1801 LGIHSAGSMG IAAASIVSQE MIRAVVNAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGKDNG

1921 RLTVKQALEG LEGMDPMDRN TSPGLPYTAL GMRRTDVVDW ESATLIPFAA ERLRKMNEGD

1981 FSEVVYQTFL KDELRPIEKV QAAKTRIVDV PPFEHCILGR QLLGKFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLTRE

2101 YLESLAISTH AFEEKRFLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLDFDKV RASLAKTGYK ITPANKTSTF PLNSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK QEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:23)

Fig. 71B

```
   1 MATTMEQETC AHSLTFEECP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS EGNEGVIINN FYSNQYQNSI DLSANAAGSD PPRTYGQFSN
 121 LFSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDTFVMD NRWSKDNLPN GARTQTNKKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPSNYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNADGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGVTENADAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF TVQSGSLESG FAPFSNKTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPTSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHVLIQ FNHRGLEVRL FRHGQFWAET RADVILRSKT KQVSFLSNGN
 961 YPSMDSRAPW NPWKNTYQAV LRAEPCRVTM DIYYKRVRPF RLPLVQKEWR VREENVFGLY
1021 RIFNAHYAGY FADLLIHDIE TNPGPFMFRP RKQVFQTQGA AVSSMAQTLL PNDLASKAMG
1081 SAFTALLDAN EDARKAMKII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI AAKFKTIFIT PPPRFPTISL FQQQSPLKQV
1201 NDFFSLAKNL DWAVKTVEKV VDWFGTWIVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMAA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV
```

Fig. 72A

1381 STTNFLPNMA SLERKGTPFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC

1441 SKTEAGYKVL DVERAFRPTG EAPLPCFQNN CLFLEKAGLQ FRDNRTKEII SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQA PVDEVSFHSV VQQLKARQEA TDEQLEELQE AFAKVQERNS

1561 VFSDWLKISA MLCAATLALS QVVKMAKAVK QMVKPDLVRV QLDEQEQGPY NETARAKPKT

1621 LQLLDIQGPN PVMDFEKYVA KHVTAPIDFV YPTGVSTQTC LLVRGRTLAV NRHMAESDWT

1681 SIVVRGVTHA RSTVKILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAG DVLPTGAAPV

1741 TGIMNTDIPM MYTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGSKKI

1801 LGIHSAGSMG IAAASIVSQE MIRAVVNAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGKDNG

1921 RLTVKQALEG LEGMDPMDRN TSPGLPYTAL GMRRTDVVDW ESATLIPFAA ERLRKMNEGD

1981 FSEVVYQTFL KDELRPIEKV QAAKTRIVDV PPFEHCILGR QLLGKFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLTRE

2101 YLESLAISTH AFEEKRFLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLDFDKV RASLAKTGYK ITPANKTSTF PLNSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK QEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:24)

Fig. 72B

```
   1 MATTMEQEIC AHSLTLKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS DGNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
 121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNVDGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF TVKSGSLESG FTPFSNQTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPTKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGITNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGMFWAEA HADVILRSRT KQISFLNNGS
 961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQTQGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIFTT PPPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV
```

Fig. 73A

1381 STTNFLPNMA SLERNGTPFT SQIVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC

1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQA PVDEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERSS

1561 VFSDWMKISA MLCAATLALS QVVKMAKTVK QMVRPDLVRV QLDEQEQGPY NEAVRAKPKT

1621 LQLLDIQGPN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS

1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPATSAPV

1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGKKKI

1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARRV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG

1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD

1981 FSDIVYQTFL KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLVKE

2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK PEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:25)

Fig. 73B

```
   1 MATTMEQEIC AHSLTFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS DGNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
 121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNVDGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF AVKSGSLESG FAPFSNETCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPAKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGMFWAEA HADVILRSRT KQISFLNNGS
 961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQTQGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIFTT PPPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV
```

Fig. 74A

1381 STTNFLPNMA SLERNGTPFT SQIVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC

1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQA PVAEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERSS

1561 VFSDWMKISA MLCAATLALS QVVKMAKTVK QMVRPDLVRV QLDEQEQGPY NEAVRAKPKT

1621 LQLLDIQGPN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS

1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPATSAPV

1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGKKKI

1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARRV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG

1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD

1981 FSDIVYQTFL KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLVKE

2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK PEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:26)

Fig. 74B

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   1 MATTMEQEIC AHSLTFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS DGNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
 121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWPVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNVDGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF TVKSGSLESG FAPFSNETCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPAKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGVSNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHVQFWAEA HADVILRSRT KQISFLNNGS
 961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 GIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQTQGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIFTT PPPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
```

Fig. 75A

1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV

1381 STTNFLPNMA SLERNGTPFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC

1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQA PVAEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERSS

1561 VFSDWMKISA MLCAATLALT QVVKMAKTVK QMVRPDLVRV QLDEQEQGPY NEAVRAKPKT

1621 LQLLDIQGPN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS

1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPATSAPV

1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGKKKI

1801 LGMHSAGSMG VAAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG

1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD

1981 FSDIVYQTFL KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLVKE

2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK PEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:27)

Fig. 75B

```
   1 MATTMEQEIC AHSLTFKGCP KCSALQYRNG FYLLKYDEEW YPEELLTDGE DDVFDPELDM
  61 EVVFELQGNS TSSDKNNSSS DGNEGVIINN FYSNQYQNSI DLSANATGSD PPRTYGQFSN
 121 LLSGAVNAFS NMIPLLADQN TEEMENLSDR VLQDTAGNTV TNTQSTVGRL VGYGAVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGAALRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDAFAMD NRWSKDNLPN GTKTQTNRKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHETL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK IPNAVPYIEA SNTAVKTQPL ATYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ VNITNVDGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE RGVTEDTDAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF TVKSGSLESG FAPFSNKTCP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNR
 721 NEETSKVFPL KSKQDYSFCL FSPFVYYKCD LEVTLSPHTS GNHGLLVRWC PTGTPAKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGISNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGSLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPSSGDKID MTPRAGVLML
 901 ESPNALDISR TYPTLHILIQ FNHGGLEIRL FRHGQFWAEA HADVILRSRT KQISFLNNGS
 961 FPSMDARAPW NPWKNTYHAV LRAEPYRVTM DVYHKRIRPF RLPLVQKEWN VREENVFGLY
1021 SIFNAHYAGY FADLLIHDIE TNPGPFMAKP KKQVFQTQGA AVSSMAQTLL PNDLASKVMG
1081 SAFTALLDAN EDAQKAMRII KTLSSLSDAW ENVKETLNNP EFWKQLLSRC VQLIAGMTIA
1141 VMHPDPLTLL CLGTLTAAEI TSQTSLCEEI VAKFKKIFTT PPPRFPTISL FQQQSPLKQV
1201 NDVFSLAKNL DWAVKTVEKV VDWFGTWVVQ EEKEQTLDQL LQRFPEHAKR ISDLRNGMSA
1261 YVECKESFDF FEKLYNQAVK EKRTGIAAVC EKFRQKHDHA TARCEPVVIV LRGDAGQGKS
1321 LSSQVIAQAV SKTIFGRQSV YSLPPDSDFF DGYENQFAAI MDDLGQNPDG SDFTTFCQMV
```

Fig. 76A

1381 STTNFLPNMA SLERKGTPFT SQLVVATTNL PEFRPVTIAH YPAVERRITF DYSVSAGPVC

1441 SKTEAGYKVL DVERAFRPTG DAPLPCFQNN CLFLEKAGLQ FRDNRTKEIL SLVDVIERAV

1501 ARIERKKKVL TTVQTLVAQA PVDEVSFHSV VQQLKARQEA TDEQLEELQE AFAKTQERSS

1561 VFSDWMKISA MLCAATLALT QVVKMAKTVK QMVRPDLVRV QLDEQEQGPY NEAVRAKPKT

1621 LQLLDIQGPN PVMDFEKYVA KFVTAPIDFV YPTGVSTQTC LLVKGRTLAV NRHMAESDWS

1681 SIVVRGVTHA RSTVRILAIA KAGKETDVSF IRLSSGPLFR DNTSKFVKAD DVLPATSAPV

1741 IGIMNTDIPM MFTGTFLKAG VSVPVETGQT FNHCIHYKAN TRKGWCGSAL LADLGGKKKI

1801 LGMHSAGSMG RTAASIVSQE MICAVVSAFE PQGALERLPD GPRIHVPRKT ALRPTVARQV

1861 FQPAYAPAVL SKFDPRTEAD VDEVAFSKHT SNQESLPPVF RMVAKEYANR VFTLLGRDNG

1921 RLTVKQALEG LEGMDPMDKN TSPGLPYTAL GMRRTDVVDW ESATLIPYAA DRLKKMNEGD

1981 FSDIVYQTFL KDELRPVEKV QAAKTRIVDV PPFEHCILGR QLLGRFASKF QTQPGLELGS

2041 AIGCDPDVHW TAFGVAMQGF ERVYDVDYSN FDSTHSVAMF RLLAEEFFTP ENGFDPLVKE

2101 YLESLAISTH AFEEKRYLIT GGLPSGCAAT SMLNTIMNNI IIRAGLYLTY KNFEFDDVKV

2161 LSYGDDLLVA TNYQLNFDKV RASLAKTGYK ITPANKTSTF PLDSTLEDVV FLKRKFKKEG

2221 PLYRPVMNRE ALEAMLSYYR PGTLSEKLTS ITMLAVHSGK PEYDRLFAPF REVGVVVPSF

2281 ESVEYRWRSL FW (SEQ ID NO:28)

Fig. 76B

```
   1 MATTMEQEIC AHSMTFEECP KCSALQYRNG FYLLKYDEEW YPEESLTDGE DDVFDPDLDM
  61 EVVFETQGNS TSSDKNNSSS EGNEGVIINN FYSNQYQNSI DLSANATGSD PPKTYGQFSN
 121 LLSGAVNAFS NMLPLLADQN TEEMENLSDR VSQDTAGNTV TNTQSTVGRL VGYGTVHDGE
 181 HPASCADTAS EKILAVERYY TFKVNDWTST QKPFEYIRIP LPHVLSGEDG GVFGATLRRH
 241 YLVKTGWRVQ VQCNASQFHA GSLLVFMAPE YPTLDVFAMD NRWSKDNLPN GTRTQTNRKG
 301 PFAMDHQNFW QWTLYPHQFL NLRTNTTVDL EVPYVNIAPT SSWTQHASWT LVIAVVAPLT
 361 YSTGASTSLD ITASIQPVRP VFNGLRHEVL SRQSPIPVTI REHAGTWYST LPDSTVPIYG
 421 KTPVAPANYM VGEYKDFLEI AQIPTFIGNK VPNAVPYIEA SNTAVKTQPL AVYQVTLSCS
 481 CLANTFLAAL SRNFAQYRGS LVYTFVFTGT AMMKGKFLIA YTPPGAGKPT SRDQAMQATY
 541 AIWDLGLNSS YSFTVPFISP THFRMVGTDQ ANITNVDGWV TVWQLTPLTY PPGCPTSAKI
 601 LTMVSAGKDF SLKMPISPAP WSPQGVENAE KGVTENTDAT ADFVAQPVYL PENQTKVAFF
 661 YDRSSPIGAF AVKSGSLESG FAPFSNKACP NSVILTPGPQ FDPAYDQLRP QRLTEIWGNG
 721 NEETSEVFPL KTKQDYSFCL FSPFVYYKCD LEVTLSPHTS GAHGLLVRWC PTGTPTKPTT
 781 QVLHEVSSLS EGRTPQVYSA GPGTSNQISF VVPYNSPLSV LPAVWYNGHK RFDNTGDLGI
 841 APNSDFGTLF FAGTKPDIKF TVYLRYKNMR VFCPRPTVFF PWPTSGDKID MTPRAGVLML
 901 ESPNPLDVSK TYPTLHILLQ FNHRGLEARI FRHGQLWAET HAEVVLRSKT KQISFLSNGS
 961 YPSMDATTPL NPWKSTYQAV LRAEPHRVTM DVYHKRIRPF RLPLVQKEWR TCEENVFGLY
1021 HVFETHYAGY FSDLLIHDVE TNPGPFTFKP RQRPVFQTQG AAVSSMAQTL LPNDLASKAM
1081 GSAFTALLDA NEDAQKAMKI IKTLSSLSDA WENVKGTLNN PEFWKQLLSR CVQLIAGMTI
1141 AVMHPDPLTL LCLGVLTAAE ITSQTSLCEE IAAKFKTIFT TPPPRFPVIS LFQQQSPLKQ
1201 VNDVFSLAKN LDWAVKTVEK VVDWFGTWVA QEEREQTLDQ LLQRFPEHAK RISDLRNGMA
1261 AYVECKESFD FFEKLYNQAV KEKRTGIAAV CEKFRQKHDH ATARCEPVVI VLRGDAGQGK
```

Fig. 77A

1321 SLSSQIIAQA VSKTIFGRQS VYSLPPDSDF FDGYENQFAA IMDDLGQNPD GSDFTTFCQM

1381 VSTTNLLPNM ASLERKGTPF TSQLVVATTN LPEFRPVTIA HYPAVERRIT FDYSVSAGPV

1441 CSKTEAGCKV LDVERAFRPT GDAPLPCFQN NCLFLEKAGL QFRDNRSKEI LSLVDVIERA

1501 VTRIERKKKV LTAVQTLVAQ GPVDEVSFYS VVQQLKARQE ATDEQLEELQ EAFARVQERS

1561 SVFSDWMKIS AMLCAATLAL TQVVKMAKAV KQMVRPDLVR VQLDEQEQGP YNETTRIKPK

1621 TLQLLDVQGP NPTMDFEKFV AKFVTAPIGF VYPTGVSTQT CLLVKGRTLA VNRHMAESDW

1681 TSIVVRGVSH TRSSVKIIAI AKAGKETDVS FIRLSSGPLF RDNTSKFVKA SDVLPHSSSP

1741 LIGIMNVDIP MMYTGTFLKA GVSVPVETGQ TFNHCIHYKA NTRKGWCGSA ILADLGGSKK

1801 ILGFHSAGSM GVAAASIISQ EMIDAVVQAF EPQGALERLP DGPRIHVPRK TALRPTVARQ

1861 VFQPAFAPAV LSKFDPRTDA DVDEVAFSKH TSNQETLPPV FRMVAREYAN RVFALLGRDN

1921 GRLSVKQALD GLEGMDPMDK NTSPGLPYTT LGMRRTDVVD WETATLIPFA AERLEKMNNK

1981 DFSDIVYQTF LKDELRPIEK VQAAKTRIVD VPPFEHCILG RQLLGKFASK FQTQPGLELG

2041 SAIGCDPDVH WTAFGVAMQG FERVYDVDYS NFDSTHSVAI FRLLAEEFFS EENGFDPLVK

2101 DYLESLAISK HAYEEKRYLI TGGLPSGCAA TSMLNTIMNN IIIRAGLYLT YKNFEFDDVK

2161 VLSYGDDLLV ATNYQLNFDR VRTSLAKTGY KITPANKTST FPLESTLEDV VFLKRKFKKE

2221 GPLYRPVMNR EALEAMLSYY RPGTLSEKLT SITMLAVHSG KQEYDRLFAP FREVGVIVPT

2281 FESVEYRWRS LFW (SEQ ID NO:29)

Fig. 77B

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   1 MACKHGYPDV CPICTAVDVT PGFEYLLLAD GEWFPTDLLC VDLDDDVFWP SNSSNQSETM
  61 EWTDLPLVRD IVMEPQGNAS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
 121 APQNNGQLSN ILGGAANAFA TMAPLLLDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
 181 CGYGEAHHGE HPASCADTAT DKVLAAERYY TIDLASWTTT QEAFSHIRIP LPHVLAGEDG
 241 GVFGATLRRH YLCKTGWRVQ VQCNASQFHA GSLLVFMAPE FYTGKGTKTG DMEPTDPFTM
 301 DTTWRAPQGA PTGYRYDSRT GFFAMNHQNQ WQWTVYPHQI LNLRTNTTVD LEVPYVNIAP
 361 TSSWTQHANW TLVVAVFSPL QYASGSSSDV QITASIQPVN PVFNGLRHET VIAQSPIAVT
 421 VREHKGCFYS TNPDTTVPIY GKTISTPNDY MCGEFSDLLE LCKLPTFLGN PNSNNKRYPY
 481 FSATNSVPTT SLVDYQVALS CSCMCNSMLA AVARNFNQYR GSLNFLFVFT GAAMVKGKFL
 541 IAYTPPGAGK PTTRDQAMQA TYAIWDLGLN SSFVFTAPFI SPTHYRQTSY TSATIASVDG
 601 WVTVWQLTPL TYPSGAPVNS DILTLVSAGD DFTLRMPISP TKWAPQGSDN AEKGKVSNDD
 661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNIES TFVYQENDLR LNCLLLTPLP
 721 SFCPDSTSGP VKTKAPVQWR WVRSGGTTNF PLMTKQDYAF LCFSPFTYYK CDLEVTVSAL
 781 GTDTVASVLR WAPTGAPADV TDQLIGYTPS LGETRNPHMW LVGAGNTQIS FVVPYNSPLS
 841 VLPAAWFNGW SDFGNTKDFG VAPNADFGRL WIQGNTSASV RIRYKKMKVF CPRPTLFFPW
 901 PVSTRSKINA DNPVPILELE NPAAFYRIDL FITFIDEFIT FDYKVHGRPV LTFRIPGFGL
 961 TPAGRMLVCM GEKPAHGPFT SSRSLYHVIF TATCSSFSFS IYKGRYRSWK KPIHDELVDR
1021 GYTTFGEFFR AVRAYHADYY KQRLIHDVEM NPGPVQSVFQ PQGAVLTKSL APQAGIQNLL
1081 LRLLGIDGDC SEVSKAITVV TDLFAAWERA KTTLVSPEFW SKLILKTTKF IAASVLYLHN
1141 PDFTTTVCLS LMTGVDLLTN DSVFDWLKNK LSSFFRTPPP VCPNVLQPQG PLREANEGFT
1201 FAKNIEWAMK TIQSIVNWLT SWFKQEEDHP QSKLDKFLME FPDHCRNIMD MRNGRKAYCE
1261 CTASFKYFDE LYNLAVTCKR IPLASLCEKF KNRHDHSVTR PEPVVVVLRG AAGQGKSVTS
1321 QIIAQSVSKM AFGRQSVYSM PPDSEYFDGY ENQFSVIMDD LGQNPDGEDF TVFCQMVSST
```

Fig. 78A

1381 NFLPNMAHLE RKGTPFTSSF IVATTNLPKF RPVTVAHYPA VDRRITFDFT VTAGPHCTTS

1441 NGMLDIEKAF DEIPGSKPQL ACFSADCPLL HKRGVMFTCN RTKAVYNLQQ VVKMVNDTIT

1501 RKTENVKKMN SLVAQSPPDW EHFENILTCL RQNNAALQDQ LDELQEAFAQ ARERSDFLSD

1561 WLKVSAIIFA GIASLSAVIK LASKFKESIW PSPVRVELSE GEQAAYAGRA RAQKQALQVL

1621 DIQGGGKVLA QAGNPVMDFE LFCAKNMVAP ITFYYPDKAE VTQSCLLLRA HLFVVNRHVA

1681 ETEWTAFKLK DVRHERDTVV TRSVNRSGAE TDLTFIKVTK GPLFKDNVNK FCSNKDDFPA

1741 RNDAVTGIMN TGLAFVYSGN FLIGNQPVNT TTGACFNHCL HYRAQTRRGW CGSAVICNVN

1801 GKKAVYGMHS AGGGGLAAAT IITRELIEAA EKSMLALEPQ GAIVDISTGS VVHVPRKTKL

1861 RRTVAHDVFQ PKFEPAVLSR YDPRTDKDVD VVAFSKHTTN MESLPPVFDI VCDEYANRVF

1921 TILGKDNGLL TVEQAVLGLP GMDPMEKDTS PGLPYTQQGL RRTDLLNFNT AKMTPQLDYA

1981 HSKLVLGVYD DVVYQSFLKD EIRPLEKIHE AKTRIVDVPP FAHCIWGRQL LGRFASKFQT

2041 KPGLELGSAI GTDPDVDWTP YAAELSGFNY VYDVDYSNFD ASHSTAMFEC LIKNFFTEQN

2101 GFDRRIAEYL RSLAVSRHAY EDRRVLIRGG LLSGCAATSM LNTIMNNVII RAALYLTYSN

2161 FEFDDIKVLS YGDDLLIGTN YQIDFNLVKE RLAPFGYKIT PANKTTTFPL TSHLQDVTFL

2221 KRRFVRFNSY LFRPQMDAVN LKAMVSYCKP GTLKEKLMSI ALLAVHSGPD IYDEIFLPFR

2281 NVGIVVPTYS SMLYRWLSLF R (SEQ ID NO:30)

Fig. 78B

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   1 MACKHGYPDV CPICTAVDAT PDFEYLLMAD GEWFPTDLLC VDLDDDVFWP SDTSTQPQTM
  61 EWTDVPLVCD TVMEPQGNAS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
 121 APQNNGQLSS ILGGAANAFA TMAPLLMDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
 181 CGYGKSHHGE HPTSCADAAT DKVLAAERYY TIDLASWTTS QEAFSHIRIP LPHVLAGEDG
 241 GVFGATLRRH YLCKTGWRVQ VQCNASQFHA GSLLVFMAPE FYTGKGTKSG TMEPSDPFTM
 301 DTTWRSPQSA PTGYRYDRQA GFFAMNHQNQ WQWTVYPHQI LNLRTNTTVD LEVPYVNVAP
 361 SSSWTQHANW TLVVAVLSPL QYATGSSPDV QITASLQPVN PVFNGLRHET VLAQSPIPVT
 421 VREHQGCFYS TNPDTTVPIY GKTISTPSDY MCGEFSDLLE LCKLPTFLGN PSTDNKRYPY
 481 FSATNSVPAT SLVDYQVALS CSCTANSMLA AVARNFNQYR GSLNFLFVFT GAAMVKGKFR
 541 IAYTPPGAGK PTTRDQAMQA TYAIWDLGLN SSFNFTAPFI SPTHYRQTSY TSPTITSVDG
 601 WVTVWQLTPL TYPSGTPTHS DILTLVSAGD DFTLRMPISP TKWVPQGIDN AEKGKVSNDD
 661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNMET TFSYQENDFR LNCLLLTPLP
 721 SYCPDSSSGP VRTKAPVQWR WVRSGGANGA NFPLMTKQDY AFLCFSPFTY YKCDLEVTVS
 781 AMGAGTVSSV LRWAPTGAPA DVTDQLIGYT PSLGETRNPH MWIVGSGNSQ ISFVVPYNSP
 841 LSVLPAAWFN GWSDFGNTKD FGVAPTSDFG RIWIQGNSSA SVRIRYKKMK VFCPRPTLFF
 901 PWPTPTTTKI NADNPVPILE LENPASLYRI DLFITFTDEL ITFDYKVHGR PVLTFRIPGF
 961 GLTPAGRMLV CMGAKPAHSP FTSSKSLYHV IFTSTCNSFS FTIYKGRYRS WKKPIHDELV
1021 DRGYTTFREF FKAVRGYHAD YYKQRLIHDV EMNPGPVQSV FQPQGAVLTK SLAPQAGIQN
1081 ILLRLLGIEG DCSEVSKAIT VVTDLVAAWE KAKTTLVSPE FWSELILKTT KFIAASVLYL
1141 HNPDFTTTVC LSLMTGVDLL TNDSVFDWLK SKLSSFFRTP PPACPNVMQP QGPLREANEG
1201 FTFAKNIEWA TKTIQSIVNW LTSWFKQEED HPQSKLDKLL MEFPDHCRNI MDMRNGRKAY
1261 CECTASFKYF DDLYNLAVTC KRIPLASLCE KFKNRHDHSV TRPEPVVAVL RGAAGQGKSV
1321 TSQIIAQSVS KMAFGRQSVY SMPPDSEYFD GYENQFSVIM DDLGQNPDGE DFTVFCQMVS
1381 STNFLPNMAH LERKGTPFTS SFIVATTNLP KFRPVTVAHY PAVDRRITFD FTVTAGPHCK
```

Fig. 79A

1441 TPAGMLDIEK AFDEIPGSKP QLACFSADCP LLHKRGVMFT CNRTKTVYNL QQVVKMVNDT

1501 ITRKTENVKK MNSLVAQSPP DWQHFENILT CLRQNNAALQ DQVDELQEAF TQARERSDFL

1561 SDWLKVSAII FAGIVSLSAV IKLASKFKES IWPTPVRVEL SEGEQAAYAG RARAQKQALQ

1621 VLDIQGGGKV LAQAGNPVMD FELFCAKNMV SPITFYYPDK AEVTQSCLLL RAHLFVVNRH

1681 VAETEWTAFK LRDVRHERDT VVMRSVNRSG AETDLTFVKV TKGPLFKDNV NKFCSNKDDF

1741 PARNDTVTGI MNTGLAFVYS GNFLIGNQPV NTTTGACFNH CLHYRAQTRR GWCGSAIICN

1801 VNGKKAVYGM HSAGGGGLAA ATIITRELIE AAEKSMLALE PQGAIVDIST GSVVHVPRKT

1861 KLRRTVAHDV FQPKFEPAVL SRYDPRTDKD VDVVAFSKHT TNMESLPPIF DIVCGEYANR

1921 VFTILGKDNG LLTVEQAVLG LSGMDPMEKD TSPGLPYTQQ GLRRTDLLDF NTAKMTPQLD

1981 YAHSKLVLGV YDDVVYQSFL KDEIRPLEKI HEAKTRIVDV PPFAHCIWGR QLLGRFASKF

2041 QTKPGFELGS AIGTDPDVDW TRYAAELSGF NYVYDVDYSN FDASHSTAMF ECLINNFFTE

2101 QNGFDRRIAE YLRSLAVSRH AYEDRRVLIR GGLPSGCAAT SMLNTIMNNV IIRAALYLTY

2161 SNFEFDDIKV LSYGDDLLIG TNYQIDFNLV KERLAPFGYK ITPANKTTTF PLTSHLQDVT

2221 FLKRRFVRFN SYLFRPQMDA VNLKAMVSYC KPGTLKEKLM SIALLAVHSG PDIYDEIFLP

2281 FRNVGIVVPT YDSMLYRWLS LFR (SEQ ID NO:31)

Fig. 79B

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   1 MACKHGYPDV CPICTAVDAT PGFEYLLMAD GEWYPTDLLC VDLDDDVFWP SDTSNQSQTM
  61 DWTDVPLIRD IVMEPQGNSS SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSASGGNAGD
 121 APQTNGQLSN ILGGAANAFA TMAPLLLDQN TEEMENLSDR VASDKAGNSA TNTQSTVGRL
 181 CGYGKSHHGE HPASCADTAT DKVLAAERYY TIDLASWTTS QEAFSHIRIP LPHVLAGEDG
 241 GVFGATLRRH YLCKTGWRVQ VQCNASQFHA GSLLVFMAPE FYTGKGTKTG TMEPSDPFTM
 301 DTEWRSPQGA PTGYRYDSRT GFFATNHQNQ WQWTVYPHQI LNLRTNTTVD LEVPYVNVAP
 361 SSSWTQHANW TLVVAVLSPL QYATGSSPDV QITASLQPVN PVFNGLRHET VIAQSPIPVT
 421 VREHKGCFYS TNPDTTVPIY GKTISTPSDY MCGEFSDLLE LCKLPTFLGN PNTNNKRYPY
 481 FSATNSVPAT SMVDYQVALS CSCMANSMLA AVARNFNQYR GSLNFLFVFT GAAMVKGKFL
 541 IAYTPPGAGK PTTRDQAMQS TYAIWDLGLN SSFNFTAPFI SPTHYRQTSY TSPTITSVDG
 601 WVTVWKLTPL TYPSGTPTNS DILTLVSAGD DFTLRMPISP TKWVPQGVDN AEKGKVSNDD
 661 ASVDFVAEPV KLPENQTRVA FFYDRAVPIG MLRPGQNMET TFNYQENDYR LNCLLLTPLP
 721 SFCPDSSSGP QKTKAPVQWR WVRSGGVNGA NFPLMTKQDY AFLCFSPFTF YKCDLEVTVS
 781 ALGMTRVASV LRWAPTGAPA DVTDQLIGYT PSLGETRNPH MWLVGAGNSQ VSFVVPYNSP
 841 LSVLPAAWFN GWSDFGNTKD FGVAPNADFG RLWIQGNTSA SVRIRYKKMK VFCPRPTLFF
 901 PWPTPTTTKI NADNPVPILE LENPAALYRI DLFITFTDEF ITFDYKVHGR PVLTFRIPGF
 961 GLTPAGRMLV CMGEQPAHGP FTSSRSLYHV IFTATCSSFS FSIYKGRYRS WKKPIHDELV
1021 DRGYTTFGEF FKAVRGYHAD YYRQRLIHDV ETNPGPVQSV FQPQGAVLTK SLAPQAGIQN
1081 LLLRLLGIDG DCSEVSKAIT VVTDLVAAWE KAKTTLVSPE FWSKLILKTT KFIAASVLYL
1141 HNPDFTTTVC LSLMTGVDLL TNDSVFDWLK QKLSSFFRTP PPACPNVMQP QGPLREANEG
1201 FTFAKNIEWA MKTIQSVVNW LTSWFKQEED HPQSKLDKLL MEFPDHCRNI MDMRNGRKAY
1261 CECTASFKYF DELYNLAVTC KRIPLASLCE KFKNRHDHSV TRPEPVVVVL RGAAGQGKSV
1321 TSQIIAQSVS KMAFGRQSVY SMPPDSEYFD GYENQFSVIM DDLGQNPDGE DFTVFCQMVS
1381 STNFLPNMAH LERKGTPFTS SFIVATTNLP KFRPVTVAHY PAVDRRITFD FTVTAGPHCK
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Fig. 80A

1441 TPAGMLDVEK AFDEIPGSKP QLACFSADCP LLHKRGVMFT CNRTQTVYNL QQVVKMVNDT

1501 ITRKTENVKK MNSLVAQSPP DWEHFENILT CLRQNNAALQ DQLDELQEAF AQARERSDFL

1561 SDWLKVSAII FAGIASLSAV IKLASKFKES IWPTPVRVEL SEGEQAAYAG RARAQKQALQ

1621 VLDIQGGGKV LAQAGNPVMD FELFCAKNIV APITFYYPDK AEVTQSCLLL RAHLFVVNRH

1681 VAETDWTAFK LKDVRHERHT VALRSVNRSG AKTDLTFIKV TKGPLFKDNV NKFCSNKDDF

1741 PARNDTVTGI MNTGLAFVYS GNFLIGNQPV NTTTGACFNH CLHYRAQTRR GWCGSAIICN

1801 VNGKKAVYGM HSAGGGGLAA ATIITKELIE AAEKSMLALE PQGAIVDIAT GSVVHVPRKT

1861 KLRRTVAHDV FQPKFEPAVL SRYDPRTDKD VDVVAFSKHT TNMESLPPIF DVVCGEYANR

1921 VFTILGKENG LLTVEQAVLG LPGMDPMEKD TSPGLPYTQQ GLRRTDLLNF ITAKMTPQLD

1981 YAHSKLVIGV YDDVVYQSFL KDEIRPIEKI HEAKTRIVDV PPFAHCIWGR QLLGRFASKF

2041 QTKPGLELGS AIGTDPDVDW TRYAVELSGF NYVYDVDYSN FDASHSTAMF ECLINNFFTE

2101 QNGFDRRIAE YLRSLAVSRH AYEDRRVLIR GGLPSGCAAT SMLNTIMNNV IIRAALYLTY

2161 SNFDFDDIKV LSYGDDLLIG TNYQIDFNLV KERLAPFGYK ITPANKTTTF PLTSHLQDVT

2221 FLKRRFVRFN SYLFRPQMDA VNLKAMVSYC KPGTLKEKLM SIALLAVHSG PDIYDEIFLP

2281 FRNVGIVVPT YSSMLYRWLS LFR (SEQ ID NO:32)

Fig. 80B

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   1 MMACIHGYPS VCPICTAIDK SSDGMYLLLA DNEWFPADLL TMDLDDDVFW PNDESDVSET
  61 MDWTDLPFIL DTIMEPQGNS TSSDKSNSQS SGNEGVIINN FYSNQYQNSI DLSANGGNAG
 121 GAPKTEGQLG NILGNAANAF STMAPLLLDQ NTEEMENLSD RVDSDKAGNS AVNTQSSVGR
 181 LCGYGMHHKG KHPASCADTA TDKVLSAERY YTIDLATWTT TLGTFSHIRI PLPHVLAGED
 241 GGVFGSTLRR HYLCKCGWRI QVQCNASQFH AGSLLVFMAP EFYTGHTPVT GTTEPATPFT
 301 MDSSWQTPQQ NPVGFRYDGR TGYFALNHQN YWQWMVYPHQ ILNLRTNTSV DLEVPFTNIA
 361 PTSSWTQHAN WTLVVAVLTP LQYAAGSATD VQITASIQPV KPVFNGLRHE AVVPQSPIPV
 421 TVREHQGTFY STNPDTTVPI YGKTIATPSD YMCGEFSDLV ELCKLPTFLG NPANTSPAGG
 481 RYPYFSATNS VPATALASYQ VALSCSCMSN SMLAAVARNF NQYRGSLNFL FVFTGTAMTK
 541 GKFLIAYTPP GAGKPTTREQ AMQATYAIWD LGLNSSYNFT VPFISPTHYR QTSYTSTSIT
 601 SVDGWLTVWQ LTPLTYPANT PPNADILTLV SAGDDFTLRM PISPTKWIPQ GVDNAEKGKV
 661 SNDDATVDFV AEPVKFPDNQ TKVSFFYDRS VPLGLLRPAQ GMEQDFAYAA NDSRANSILL
 721 TPLPSYAPDS TTGPTETQAP IQWRWLRGTS DGSTTFPLMT KQDYAFLLFS PFTYYKADLE
 781 VTLSAISNSN NVTVVRWAPT GAPADISRQL SGYTPSIGDT RDPHLWFVGA GNSQTSFVVP
 841 YNSPLSVLPA AWFNGWSDFG NTKDFGVAPN ADFGRLWIQG NTSVAVRVRY KKMKVFCPRP
 901 TLFLPWPSTT TTRIHADNPV SVMELQNPFS FYRVDLFITF TDELITFDYK VHGRPVLQYQ
 961 VPGLGLTCAG RMLVCMGQMP NHAPFSTVRH LYHVVFTGSR NSFGVVIYYK RHRPWKKPLH
1021 EELHDYGFEC FSDFFKHVRE YHAAYYKQRL MHDVETNPGP PVQSVFRPQG GVLTKSQAPM
1081 SGIQNLFLRA LGIDADHGEF TRAVTMITDL CNTWEKAKNT LVSPEFWTVL IMKTVKFIAA
1141 SVLYLHNPDL TATICLSLMT GVDVLTNESI FNWLSNKLSK LFHTPPPPTS PLLQAQSPLR
1201 EANDGFNLAK NIEWAIKTVQ KIVDWLMSWF KQEEAHPQAK LDKMLADFPE HCASILAMRN
1261 GRKAYTDCAG AFKYFEDLYN LAVQCKRIPL ATLCEKFKNK HDHAVARPEP VVVVLRGNAG
1321 QGKSVTSQII AQAVSKLAFG RQSVYSIPPD SDYLDGYENQ FSVIMDDLGQ NPDGEDFKVF
1381 CQMVSSTNFL PNMAHLEKKG TPFTSNFIVA TTNLPKFRPV TVAHYPAVDR RITFDLTVEA
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Fig. 81A

1441 GPACKTPTGM LDVEKAFQEI PGEPQLDCFS SDCALLHKRG VQFICNRTKK IYNLQQIVKM

1501 VKDTIDNKVA NLKKMNTLVA QSPNNGNDME HIITCLRQNN AALQDQIDEL QEAFAQAQER

1561 QNFLSDWMKV SAIIFAGIAS LSAVCKLVGR LKNLIWPSPV HVELSEGEQA AYAGAKRGAK

1621 QALQVLDLQG GGRIIAQAGN PVMDYEVCVA KNMVAPITFY YADKAQVTQS CLLVKGRLFV

1681 VNRHVAETDW VSFELRDVRH ERDTVTMRSV NRSGMEVDLT FIKVTKGPLF KDNTKKFCSN

1741 KDDFPQKNET VTGIMNTGLP FVFNGKFIIG NHPVNTTTGA TFNHCLHYRA NTRRGWCGSA

1801 VICQVNGKKA VYGMHSAGGG GLAAATIITQ ELVEAAEQNM DRLVPQGAIM EIGTGSVVHV

1861 PRKTKLRRTV AHEIFLPKFE PAVLSRYDPR TEKDVDQVAF SKHTTNMEEL PAVFSMVAKE

1921 YANRVFTKLG KENQLLTTQQ AILGLPGMDP MEKDTSPGLP YTQQGLRRTD LVNFETGKMD

1981 HNLDYAHSKL MLGHYEDVVY QSFLKDEIRP IEKIHEAKTR IVDVPPFHHC IWGRQLLGRF

2041 ASRFQTNPGL DLGSAIGTDP DVDWTVFAHQ LAEFKYIYDV DYSNFDASHS TAIFEILIQE

2101 FFTPQNGFDP RIGEYLRSLA VSRHAYEDRR VLIRGGLPSG CAATSMINTI INNIVIRAAL

2161 YMTYANFEFD DIKVLSYGDD LLIATNYEIN FNLVKERLAP FNYKITPANK TSTFPQTSHL

2221 QDVVFLKRRF VQFNSFLFRP QMETENLKAM VSYCRPGVLK EKLMSIALLA VHSGPDVYDE

2281 IFMPFRRIGV VVPEYSTMLY RWLNLFR (SEQ ID NO:33)

Fig. 81B

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   1 MACKHGYPDV CPICTAIDVT PGFEYLLLAD GEWFPTDLLC VDLDDDVFWP SDSSNQSQTM
  61 EWTDIPLICD TVMEPQGNST SSDKSNSQSS GNEGVIINNF YSNQYQNSID LSANGGNAGD
 121 GPKTEGQLSN ILGGAANAFA TMAPLLLDEN TEEMENLSDR VDSDKAGNSA TNTQSSVGRL
 181 HGYGATHRGD HPASCADTAT DKVLAAERYY TIDLATWTTA QTTFSHIRVP LPHALAGEHG
 241 GVFGATLRRH YLAKCGWRVQ VQCNASQFHA GSLLVFLAPE FYTGTGVATS GQEPNKVFLM
 301 DTTWQEPQAA PTGFRYDGKN GFFTLNHQNY WQWTVYPHQI LNLRTNTSVD LEVPYVNVAP
 361 TSSWTQHANW ALVVAVLTPL QYSTGAATDV AITVSLQPVN PVFNGLRHEA QVPQSPVAVT
 421 VREHQGSFYS TNPDTTVPIY GKTIVTPSDY MCGEFTDLLE LCKLPTFLGN LSNDTRVPFF
 481 TATNSVPTES LVEYQVTLSC SCMSNSMLAS VARNFNQYRG SLNFLFVFTG SAMTKGKFLI
 541 AYTPPGAGKP TTRDQAXQST YAIWDLGLNS SYNFTVPFIS PSHYRQTSYT SPSIAAVDGW
 601 LTVWQLTPLT FPANVPPSSD ILTLVSAGND FTLRMPISPT KWIPQGVDNA EKGKVSDDNA
 661 SVDFVAEPIK LPENQTRVNF FYDRSSPIGL LRPNQAIESN FSYSADSNGA TNCALLTPLP
 721 SYSPDRPGQS PDTSKAPIQW RWISAVTESG TVSNTFPTRT RQDYAFLLFS PFTYYKCDLE
 781 VTLSSVGNGV VASLVRWAPT GAPADITTQL TTSTPSIGDT RDPHMWLVGA GNSQTSFVIP
 841 YNSPLSVLPA AWFNGWSNFS NTYDFGIAPC SDFGRLWIQG NAPLAIRVRY KKMRVFCPRP
 901 TLFFPWPTPT TTKVNADNPV PILDLENPAA (SEQ ID NO:34)
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Fig. 82